DESIGN, SYNTHESIS OF SOME NOVEL CINNOLINE DERIVATIVES AND SCREENING FOR ITS ANTI-MALARIAL, ANTI-TUBERCULAR AND ANTI-MICROBIAL ACTIVITY

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R Medical University, Chennai

In partial fulfillment for the requirement of the Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

April - 2012

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPATTI ROAD,

COIMBATORE 641-048.

DESIGN, SYNTHESIS OF SOME NOVEL CINNOLINE DERIVATIVES AND SCREENING FOR ITS ANTI-MALARIAL, ANTITUBERCULAR AND ANTI-MICROBIAL ACTIVITY

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R Medical University, Chennai

In partial fulfillment for the requirement of the Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

Submitted by

T.NILOFER NISHA

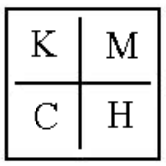
Under the guidance of

Mrs. S. HURMATH UNNISSA, M. Pharm.

Assistant Professor,

Department of Pharmaceutical Chemistry

April-2012



DEPARTMENT OF PHARMACEUTICAL CHEMISTRY,
KMCH COLLEGE OF PHARMACY
KOVAI ESTATE, KALAPATTI ROAD,
COIMBATORE 641-048

Certificate

Dr. A Rajasekaran, M. Pharm., Ph.D.,

Principal,

KMCH College of Pharmacy,

Kovai Estate, Kalapatti Road,

Coimbatore-641 048. (T.N)

CERTIFICATE

This is to certify that the dissertation work entitled ***"Design , Synthesis of some Novel Cinnoline derivatives and Screening for its Anti-malarial, Anti-tubercular and Anti-microbial activity"*** submitted by Ms.NILOFER NISHA.T is a bonafide work carried out by the candidate under the guidance of **Mrs. S.HURMATH UNNISSA, M.Pharm.** Asst Professor, to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of Master of Pharmacy in Pharmaceutical Chemistry at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

Dr. A. Rajasekaran, M.Pharm., Ph.D.

Principal

Mrs. S. HURMATH UNNISSA, M.Pharm.,

Asst.Professor,

KMCH College of Pharmacy,

Kovai Estate, Kalapatti Road,

Coimbatore 641 048(T.N)

CERTIFICATE

This is to certify that the dissertation work entitled ***"Design, Synthesis of some Novel Cinnoline derivatives and Screening for its Anti-malarial, Anti-tubercular and Anti-microbial activity"*** submitted by **Ms.NILOFER NISHA.T** under my guidance to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of Master of Pharmacy in Pharmaceutical Chemistry at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

Mrs. S. HURMATH UNNISSA, M.Pharm.,

Asst.Professor,

Dept of Pharmaceutical Chemistry.

DECLARATION

I do hereby declare that the dissertation work entitled ***"Design, Synthesis of some Novel Cinnoline derivatives and Screening for its Anti-malarial, Anti-tubercular and Anti-microbial activity"*** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of Master of Pharmacy in Pharmaceutical Chemistry at the Department of Pharmaceutical Chemistry was done by me under the guidance of **Mrs. S.HURMATH UNNISSA**, M.Pharm. Asst.Professor at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

NILOFER NISHA.T

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled ***"Design, Synthesis of some Novel Cinnoline derivatives and Screening for its Anti-malarial, Anti-tubercular and Anti-microbial activity"*** submitted by **Ms. NILOFER NISHA.T, (Reg.No. 26107134)** to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of Master of Pharmacy in Pharmaceutical Chemistry is a bonafide work carried out by the candidate at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2011-2012.

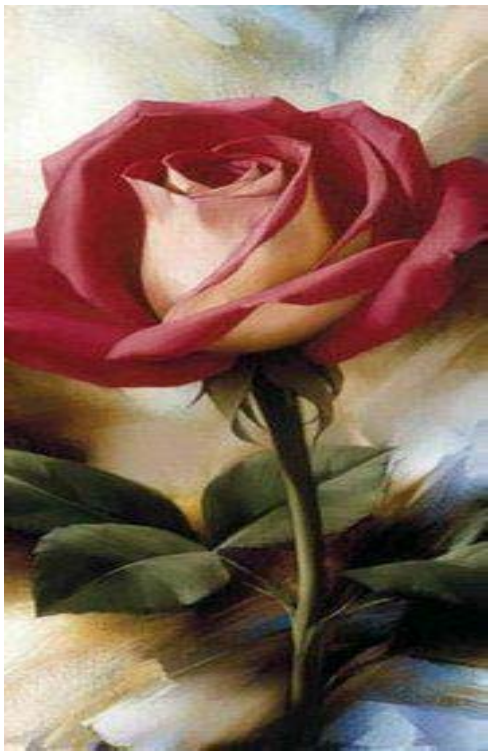
Internal Examiner **External Examiner**

Convener of Examinations

Examination Center : KMCH College of Pharmacy,

Coimbatore.

Date :



Dedicated To

My Parents

&

Almighty

Acknowledgement

ACKNOWLEDGEMENT

"Though gratitude comes deep from the heart, if left unexpressed loses its

memory, charm and above all ,the biggest asset, its beauty"

First and foremost I express my wholehearted gratitude to my esteemed guide, ***Mrs. S.Hurmath Unnissa M.Pharm.,*** Asst Professor, Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, for her constant insight, personal advice, countless serenity, expert guidance and pain taking effort for the successful completion of this work.

With great pleasure I wish to place my indebtedness to Principal ***Dr. A. Rajasekaran, M. Pharm., Ph.D.,*** for his support and for giving me an opportunity to do my project work.

My respectful regards to our beloved Managing trustee ***Dr.Thavamani.D. Palanisamy*** and our respected Chairman *Dr.Nalla.G.Palanisamy*, KMCH College of Pharmacy, Coimbatore.

I express my special thanks to Mr***. K.Suresh Kumar, M.Pharm, Ph.D., Mr.I.Ponnilavarasan***, ***M.Pharm***, Department of Pharmaceutical chemistry for his encouragement he rendered during his work.

My heartful thanks to ***Mr. Siva*** and ***Mr. R.S. Shanmugarajan*** for providing me the malarial blood samples for performing anti-malarial activity.

My Special thanks to ***Mr.Leishmann***, Department of microbiology, Madurai Kamaraj university, ***Astrazenica India Pvt Ltd***., Bangalore and ***IIT Madras***, for their timely help for the biological and analytical studies.

I convey my sincere thanks to ***Mr. Sundaramurthi, M.Pharm***. HOD, Department of Pharmaceutical biotechnology, ***Dr.Adhirajan, M.Pharm., Ph.D***., Dept. of Pharmaceutical biotechnology for the encouragement they have given me for my dissertation work.

I take this opportunity with pride and immense pleasure to thank all my teachers who lit the light of knowledge and wisdom in my life. I also acknowledge the support of all lab assistants and the library staffs.

This project would not be a resplendent one without the timely help and continuous support of all my classmates especially my dearest friend ***S.Saranya*** and my mother ***Mrs.T.Shamila*** for the memorable company, co-operation and prayers offered to me.

I express my heartful thanks to ***Mr. Mohd Mohiuddin Ahmed***, for his support and encouragement. His encouragement was highly inspirational throughout the course of this work.

Above all I dedicate myself before the unfailing presence of God, and constant love and encouragement given to me by my beloved ***Attha-M.Thasthakir, Amma-T.Shamila, Uncle-M.Y.Ahmed, Ammy-Ifroze faizia, Grandma-Jamruth beevi, Brothers*** and all my family members who deserve the credit of success in whatever work I did.

Last but not least, I would like to thank everyone who was important to the successful realization of the thesis.

Content

CONTENTS

\

LIST OF TABLES

ABBREVIATIONS USED

DMSO - Dimethyl Sulfoxide

FTIR - Fourier Transform Infrared Spectrometer

IR - Infrared Spectral analysis

TLC - Thin Layer Chromatography

UV - Ultraviolet and Visible Spectroscopy

NMR - Nuclear Magnetic Resonance

IC50 - Inhibitory Concentration Percentage

Rif - Rifampicin

Std - Standard

Mins - Minutes

Hrs - Hours

Mg - Milligram

Ml - Millilitre

Mm - Millimetre

µg - Microgram

δ - Delta

λ - Lambda

0C - Degree Celsius

% - Percenta**ge**

α - Alpha

β - Beta

COMPOUND CODE

S.No	Compound code	Name of the compound
1	CN-I	4-methyl-3-acetylcinnoline-6-sulfonamide
2	CN-1a	4-methyl-3-[5-(4-nitrophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide
3	CN-2a	4-methyl-3-[5-(4-hydroxyphenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide
4	CN-3a	4-methyl-3-[5-(4-chlorophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide
5	CN-4a	4-methyl-3-[5-(4-methoxyphenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide
6	CN-5a	4-methyl-3-[5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide
7	CN-6a	4-methyl-3-{5-[(E)-2-phenylethenyl)-4,5-dihydro-1H-Pyrazol-3-yl}cinnoline-6-sulfonamide
8	CN-7a	4-methyl-3-[5-(4-dimethylphenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide
9	CN-8a	4-methyl-3-[5-(3-fluorophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide
10	CN-9a	4-methyl-3-[5-(2-chlorophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide
11	CN-10a	4-methyl-3-[5-(3-chlorophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide
12	CN-11a	4-methyl-3-[5-(2-nitrophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-sulfonamide

Introduction

INTRODUCTION

In the ancient times, people obtained medicines to cure illness from nature. The rising number of diseases and the emergence of resistance to established therapies gradually raised the need for the discovery and development of new drug moieties.

This thesis deals on the synthesis, characterization and screening of some novel Cinnoline derivatives. This chapter deals with the importance of heterocyclic compounds, chemistry of Cinnoline and brief introduction to therapeutic activity based on the ring. A literature survey on the investigation carried out by earlier workers on this ring moiety is also included in this chapter.

Medicinal chemists[1-2] has stimulated by the need of sick for drugs, have made some of the proudest and most spectacular achievements of contributing therapeutic agents. It have been started that 'Medicinal chemistry' concerns the discovery, the development, identification and interpretation, mode of action of biologically active compounds at the molecular level. The primary objective of medicinal chemistry is the design and discovery of new moieties that are suitable for use as drugs. The discovery of a new drug involves not only its design and synthesis but also to establish how a new drug entity elicits its action into the body. Drug discovery may also require fundamental research into the biological and chemical nature of the diseased state.

The knowledge required by a medicinal chemist is both a challenge and a reward. His work is centered on the discovery of new molecular soldiers to win the battle against disease. It includes the development of more effective and safer analogues from both, new and existing lead compounds. This usually involves synthesizing and testing many hundreds of compounds before a suitable compound is produced.

Heterocyclic rings[3-6], which have been reason for the activity of most of the drugs of natural origin leads to the discovery of many synthetic drugs possessing the heterocyclic rings and their fused analogs represent an important class of heterocyclic compounds exists in numerous natural products displaying a wide range of biological and pharmaceutical activities. On intensive research heterocyclic derivaties continue to yield new medicinal agents.

MALARIA[56] is a mosquito borne infectious disease caused by a eukaryotic protest of genus Plasmodium. It have been one of the greatest burdens to mankind, with a mortality rate which is unmatched by any other modern diseases, this is the major health problem in most of the countries in the tropics. Malaria affects more than 2400 million people, over 40% of the world's population, in more than 100 countries in the tropics from South America to Indian peninsula. The tropics provides an ideal breeding and

living conditions of the anopheles mosquito, and hence this distribution. Over the years, as per statistical records, it have been estimated that there may be 300 to 500 million new infection and 1 to 3 million infection related to the deaths annually caused by malaria, and it have also been found that more than 90% of the deaths occur in the regions in and around Sub-Saharan Africa. Malaria kills in 1 year what AIDS killed in 155 year, if 5 million people have died of AIDS, 50 million people have died of malaria.

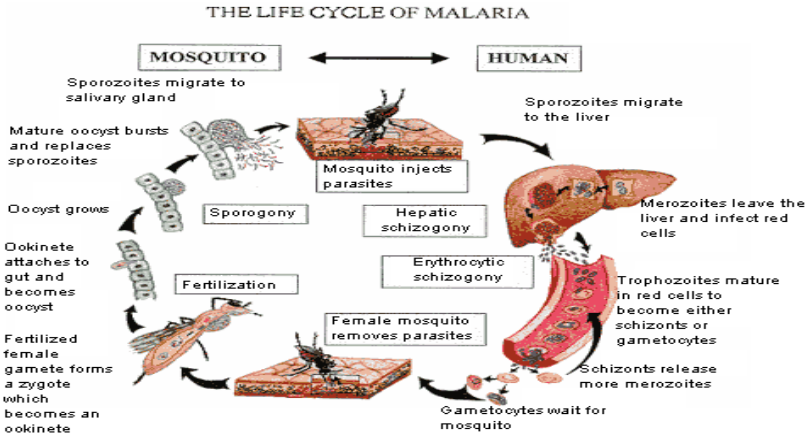
Yet, in this age of tremendous technological and medical advancements, its a big surprise that human knowledge power have not been able to control this disease, if not eradicate it. The intolerable impact of malaria have been sometimes fully or partially attributed to their increasing resistance of the Plasmodium parasite to chemoprophylactic and chemotherapeutic agents, and their resistance of the Anopheles species mosquito vector to insecticides, including the pyrethroids used in insecticide impregnated bednets. Adding to these its the inability of health, public and civil work departments of the affected countries in mobilizing and sustaining the resources required to malarial control.

Malaria is caused by Plasmodium parasites[57, 58]:

Malaria is one of the most widespread infectious diseases of our time; it belongs to the genus Plasmodium. There are five species of the protozoan parasites which infect humans. Malaria is caused by the Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale and Plasmodium knowlesi.

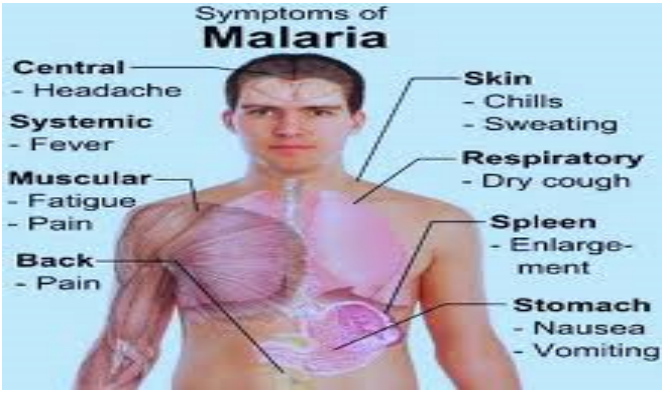
Life cycle of the malarial parasite:

The life cycle of the malarial parasite is complicated, which involves two hosts, human and Anopheles mosquito. Plasmodium falciparum and, to a much lesser extent, P.vivax are the main causes of disease and death due to malaria.



- A female Anopheles mosquito carrying malaria causing parasites feeds on human and injects the parasites in the form of sporozoites into their blood stream. The sporozoites travel in to their liver and invade liver cells.
- Over 5-16 days, their sporozoites grow, divide, which produce tens of thousands of haploid forms called merozoites, perliver cell. Some malarial parasite species remains dormant for extended periods to the liver, causing relapses weeks or months later.
- The merozoites exit in their liver cells and re-enter the blood stream, beginning the cycle for the invasion of the red blood cells, asexual replication, and release of newly formed merozoites from the red blood cells repeatedly over 1-3 days. This multiplication can result in the thousands of parasite-infected cells in their host blood stream, leading to illness and complications of malaria that can last for months if not treated.
- Some of their merozoites infected blood cells leave the cycle for asexual multiplication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasite, called male and female gametocytes which are circulate in to their blood stream.
- When a mosquito bites an infected human, it ingests the gametocytes. In the mosquito gut, the infected human blood cells burst, releasing the gametocytes, which develops further into the mature sex cells called gametes. Male and female gametes fuse to form the diploid zygotes, which develop into the actively moving ookinetes that burrow into the mosquito midgut wall and form oocysts.
- Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After 8-15 days, the oocyst bursts, releasing sporozoites into the body cavity of the mosquito, from which they travel into and invade the mosquito salivary glands. The cycles of human infection re-starts when the mosquito takes a blood meal, injecting their sporozoites from its salivary glands into the blood stream.

Symptoms of malaria[59]:



Malaria is generally characterized by recurrent attacks, each of which occurs in three stages. Moderate to severe shaking chills (cold stage) followed by high fever (hot stage), and then profuse sweating the body temperature falls (sweating stage); the classical malarial attacks lasts for 6-10 hours. Along with chills, the person is likely to have fever and flu like symptoms, such as chills, headache, muscle aches, tiredness, nausea, vomiting and diarrhoea. Malaria can also cause anaemia and jaundice due to loss of red blood cells. Malarial signs and symptoms[60] typically begins within the few weeks (10-16 days) after a bite from an infected mosquito. However, some types of malarial parasites can lie dormant in your body for months, or even years.

Diagnosis[61]:

The diagnosis of malaria is confirmed by the blood tests which can be divided into microscopic and non-microscopic tests.

- Microscopic tests involve staining and direct visualization of the parasite under the microscope.
  - Peripheral Smear study- MP test
  - Quantitative Buffy coat (QBC) test
- Non-Microscopic tests involve the identification of the parasitic antigen or the antiplasmodial antibodies or the parasitic metabolic products.
  - Rapid Diagnostic tests (RDTS)

TUBERCULOSIS:

Tuberculosis (TB) is an infectious disease caused by the bacteria whose scientific name is *Mycobacterium tuberculosis*. It was first isolated in 1882 by a German physician named Robert Koch who received the Nobel Prize for this discovery. It affects[62] 32% of the human population and it causes 1.8 million of deaths annually. As per WHO estimates, 9 million people globally develop active TB and 1.7 million people die annually. (TB) the most commonly affects the lungs but also it can involve almost any organs of the body. Many years ago, this disease was referred to as ‘consumtion’ because without effective treatment, those patients often would waste away. Today of course, tuberculosis was usually can be treated successfully with the antibiotics.

From the chemotherapeutic point of view[63], there are two sources of new chemical entities. The first is the extraordinary diversity provided by the natural products. The second results from the design of new or the modernization of synthetic transformations. Although many compounds are in clinical trials, it is

astonishing that with these backgrounds, there had been no new drugs registered to treat TB in the last 40 years. This reflects that the inherent difficulties for discovery and clinical testing of new agents and the lack of pharmaceutical industry research in this area.

In terms of organ development, tuberculosis is often divided into pulmonary (respiratory) and extra pulmonary (non respiratory) types. The later should only be applied by organ involvement, which is secondary to a pulmonary lesion. If the disease does not involve the lung, it is referred to as the non-pulmonary.

Causative Organism:

Tuberculosis[64] is an infection caused by the rod-shaped, non-spore forming, aerobic bacterium *Mycobacterium tuberculosis.* Mycobacteria typically measures upto 0.5 μm to 3 μm, which are classified to as acid-fast bacilli, and have a unique cell wall structure crucial to their survival. The well developed cell wall contains a considerable amount of the fatty acids, mycolic acid, covalently attached to their underlying peptidoglycan bound polysaccharide arabinogalactan, providing an extraordinary lipid barrier. This barrier is responsible for the many of the medically challenging physiological characteristics of tuberculosis, including resistance to antibiotics and with the host defence mechanisms. The composition and quantity of their cell wall components affects the bacteria's virulence and growth rate.

The *M.tuberculosis* complex[65] includes other TB causing mycobacteria they are *M.bovis, M.africanum, M.canetti, M.microti.*TB other known pathogenic mycobacteria include *M.leprae, M.avium, and M.kansasii.*

Mechanism[66]:

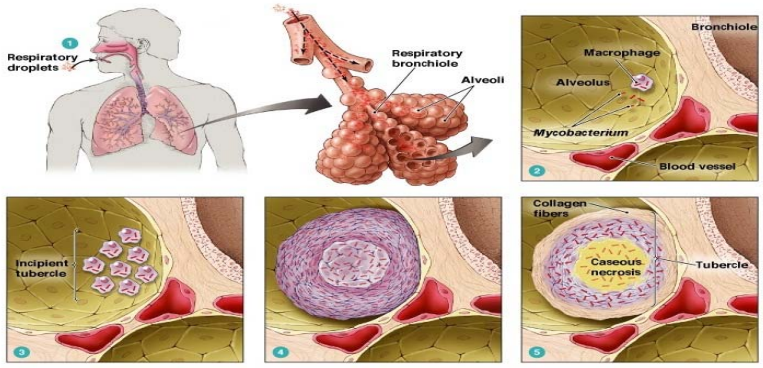
TB infection begins with when the mycobacterium reaches the pulmonary alveoli, where they invade and replicate within the endosomes of the alveolar macrophages. The primary site of infection to the lungs is called the ghon focus, and it is generally located in either the upper part of the lower lobe. Bacteria are picked up by dentritic cells, which do not allow replication, although these cells can transport the bacilli to their local lymph nodes. Further spread is through their blood stream to the other tissues and organs where the secondary tuberculosis lesions can develop in other parts of the lung, peripheral lymph nodes, kidneys, brain and bone. The severe form of tuberculosis disease was most common in infants and elderly and it is called military tuberculosis. If it's untreated, infection with *Mycobacterium tuberculosis* would become lobar pneumonia.

Pathophysiology[67-73]:

Once inhaled, the infectious droplets settled throughout their airways. The majority of the bacilli are trapped in the upper parts of the airways where their mucus secreting goblet cells exist. The mucus produced catches foreign substances, and their cilia on the surface of the cells constantly beat the mucus and its entrapped particles upward for its removal. This system provides the body with an initial physical defense, which prevents the infection in most persons exposed to tuberculosis.

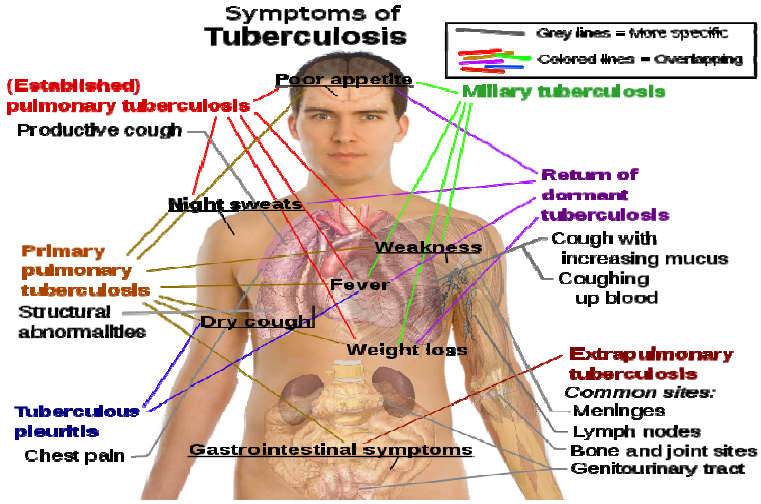
Bacteria in droplets that bypass the mucociliary system and reach the alveoli are quickly surrounded and engulfed by alveolar macrophages, the most abundant immune effector cells which are present in the alveolar spaces. These macrophages, the next line of the host defense, are the part of the innate immune system and provide an opportunity for the body to destroy the invading mycobacteria and prevent infection. Macrophages are readily available phagocytic cells that combat many pathogens without requiring previous exposure to the pathogens. The complement system also plays a major role in the phagocytosis of the bacteria. The complement protein C3 binds to the cell wall which enhances recognition of mycobacteria by macrophages. Opsonization by C3 is rapid, even in the air spaces of a host with no previous exposure to *Mycobacterium tuberculosis.*

After being ingested by macrophages, the mycobacteria continue to multiply slowly, with their bacterial cell division occurring every 25 to 32 hours. Regardless of whether the infection becomes controlled or progresses, initial development involves the production of proteolytic enzymes and cytokines by macrophages in an attempt to degrade their bacteria. Released cytokines attract the T-lymphocytes in to their site, the cells that constitute the cell-mediated immunity. Macrophages then present mycobacterial antigens to their surface of the T cells. This initial immune process continues for 2 to 12 weeks, then the microorganisms continues to grow until they reach sufficient numbers to fully elicit the cell-mediated immune response, which can be detected by a skin test.



SYMPTOMS[74]:

TB infection usually occurs in the upper part (lobe) of the lungs. The body's immune system cannot contain the TB bacteria, the bacteria will reproduce (become active or reactivate) in the lungs spread elsewhere in the body. *Mycobacterium tuberculosis* is spread by small airborne droplets, called droplet nuclei, generated by the coughing, sneezing, talking, or singing of a person with the pulmonary or laryngeal tuberculosis. TB infection leads to tiredness, weight loss, fever, night sweats and if its worsen it can cause chest pain, coughing up of sputum, shortness of breath. Introduction of *Mycobacterium tuberculosis* into the lungs leads to infection of the respiratory system; however, these organisms can spread to the other organs, such as the lymphatics, pleura, bones/joints, or meninges and cause extrapulmonary tuberculosis.



CHEMOTHERAPY[75]:

Chemotherapy is the keystone of the management of all the types of tuberculosis in man. Since Koch announced their discovery of the tubercle bacilli, many attempts had been made to find suitable therapeutic agents for the disease.

The choice of therapy should be guided by several well established principles.

- Drugs should be choosen to which the bacilli are likely to be susceptible.
- Even in generally susceptible population of bacilli, a natural resistant mutant occurs about once in 10^5 to 10^6 organisms. For this reason atleast two effective drugs should always be given to the patients with clinical tuberculosis to avoid multiplication of drug resistant mutants.
- Therapy should be continued long enough to eradicate the bacilli from the body.

- All the medication must be given in a single dose, before for maximum effect on the bacilli.
- Bacterial drugs must be prefused.

According to their clinical utility, anti-tuberculosis drugs can be divided into,

First line drugs:

These drugs have high anti-tuberculosis efficacy as well as low toxicity.

1. Isoniazid (H) 2. Rifampin (R) 3. Pyrazinamide (z) 4. Ethambutol (E) 5. Streptomycin (S)

Second line drugs:

These drugs have either low anti-tuberculosis efficacy or high toxicity or both. These are used for the special circumstances only.

1. Thiacetazone (Tzn) 2. Para amino salicylic acid (PAS) 3. Ethionamide (Etm) 4. Cycloserine (Cys)

5. Kanamycin (Kmc) 6. Amikacin (Am) 7. Capreomycin (cpr)

Newer drugs:

1. Ciprofloxacin 2. Oflaxacin 3. Clarithromycin 4. Azithromycin 5. Rifabutin

Short Course Chemotherapy:

These are the regimens of 6-9 months duration which has been found to be highly efficacious. All regimens have an initial intensive phase lasting 2-3 months aimed to rapidly kill the bacilli, bring about sputum conversion and afford symptom relief. This is followed by a continuation phase lasting 4-6 months during which the remaining bacilli are eliminated so that relapse does not occur.

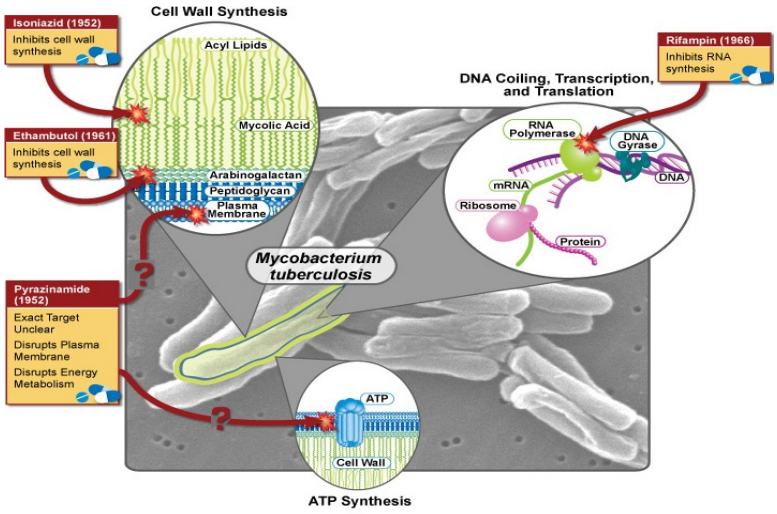
Diagnosis[76]:

- Tuberculin skin test (Mantoux test) .
- Physical examination, a chest X-ray, microbiological smears, and cultures.
- Mycobacterium tuberculosis (stained red) in the sputum.
- Polymerase chain reaction assays for the detection of bacterial DNA.

- Interferon release assays (IGRA'S)

Treatment for Tuberculosis:

Standard "short" course treatment for TB consists of a six-month regimen (two months treatment with isoniazid, rifampin and pyrazinamide followed by four months of isoniazid and rifampin). Ethambutol or streptomycin may be added based on the drug sensitivity of the patient. Multiple drug therapy is adopted due to the rapid development of resistance to single drug regimen. For latent tuberculosis, the standard treatment of six to nine months of isoniazid alone is preferred.



Mechanism of action of the first line drug used in TB

Current Knowledge about TB Research

Several strategies are now being adopted by the researchers for their discovery of new anti-tuberculosis agents which includes the large scale screening of both natural and synthetic compounds, targeting the specificity of activity, which means the inhibiting of *Tuberculosis bacillus* but not the mammalian cells lead compounds are then optimizing and also attempts are made to optimize the anti-tubercular activity of antibiotics used for the other infections.

Since the current diagnostic tools available to identify active tuberculosis are slow and unreliable, the development of newer diagnostic tools which are rapid and with improved reliability can be done as a part of the improvement of therapy. A major area of emphasis for further generation in the research to support their development of vaccines for the prevention of the tuberculosis infection. The ongoing efforts of the medicinal chemists will be surely be a greatest contribution to the community against tuberculosis.

An **anti-microbial**[77] is a substance which kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans. Anti-microbial drugs either kill microbes or prevent the growth of the microbes.

The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time the reason was one bacterium failed to grow, was that the other bacterium were producing an antibiotic. Ofcourse, in today's common usage, the term antibiotic is used to almost any drug attempts to rid of your body of the bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.

However, with the development of antimicrobials, microorganisms has adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology were based either poisons or heavy metals, which may not have killed the microbes completely, allowing the microbes to survive, change, and become resistant to the poisons and/or heavy metals.

Antimicrobial nanotechnology is a recent addition to fight against disease causing organism, replacing heavy metals and toxins and may someother day to be a viable alternative.

Mechanisms:

The mechanisms by which the microorganism exhibit resistance to the antimicrobials are,

- Drug inactivation or modification; for example, enzymatic deactivation of penicillin G in some penicillin-resistant bacteria through the production of β-lactamases.
- Alteration of target site: for example, alteration of PBP the binding target site of penicillins in MRSA and other penicillin-resistant bacteria.
- Alteration of metabolic pathway; for example, some sulfonnamide resistant bacteria do not require para-aminobenzoic acid (PABA), it is an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by the sulphonamides, instead, like mammalian cells, which turn to using performed folic acids.
- Reduced drug accumulation by decreasing drug permeability and/or increasing active efflux (pumping out) of these drugs across the cell surface.

Antimicrobial Resistance:

Antimicrobial resistance (AMR) is the resistance of a microorganism to an antimicrobial medicine to which it were previously sensitive. Resistant organisms (they include bacteria, viruses and some parasites) are able to withstand attack by antimicrobial medicines, such as antibiotics, antivirals and antimalarials. So that the standard treatments become ineffective and infections persist and may spread to others. AMR is a consequence of the use, particularly the misuse of antimicrobial medicines and develops when a microorganism mutates or acquires a resistant gene.

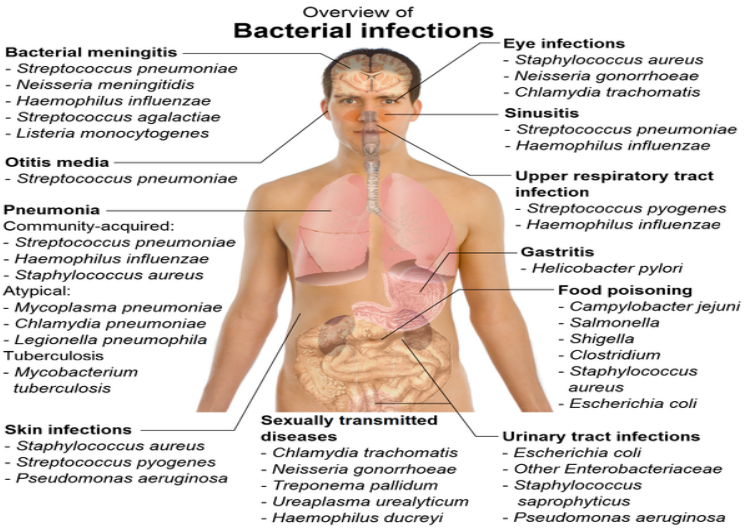
Bacteria[78] are a large domain of prokaryotic microorganisms. The bacterial cells are about one tenth the sizes of the cells and are typically 0.5-5.0 micrometres in length. It is present in most habitats on earth, growing in soil, acidic hot springs, radioactive waste, water, and deep in the earth's crust, as well as in the organic matter and the live bodies of plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a millilitre of fresh water. Bacteria are vital in recycling nutrients, with many steps in nutrient cycle depending on these organisms such as the fixation of nitrogen from the atmosphere, Putrefaction.

Bacterial infections[79, 80] may be treated with antibiotics, which are classified as bacteriocidal (kill bacteria), or bacteriostatic (prevent bacterial growth). There are many types of antibiotics and each class inhibits a process which is different in the pathogen from that found in the host. Antibiotics are used both in treating human disease and intensive farming to promote animal growth, where they may be contributing to the rapid development of antibiotic resistance to the bacterial population.

Pathogenic bacteria[81]:

Bacteria form a parasitic association with other organisms, which are classed as pathogens. Bacteria that can cause disease are called pathogenic bacteria. Bacteria can cause diseases in humans in other animals, and also in plants. Some bacteria can only make one particular host ill, depending on the host specificity of the bacteria and some pathogenic bacteria have received disproportionate attention in the press, example; the flesh eating bacteria. There are major cause of human death and disease and the infections such as tetanus, typhoid fever, diphtheria, syphilis, cholera, foodborne illness and leprosy. A pathogenic cause of a known medical disease may only be discovered many years after, as were the case with Helicobacter pylori and Peptic ulcer disease. Each species of pathogen have a characteristic spectrum of interactions with its human hosts. The organisms such as *staphylococcus* or *streptococcus,* it can cause skin infections.

Yet these organisms are also a major part of the normal human flora and usually exist on the skin or nose without causing any disease at all. Other organisms which in variably can cause disease in humans, such as the rickettsia, that are obligate intercellular parasites which is able to grow and reproduce only within the cells of other organisms.



A **Fungus**[82] is a member of a large group of eukaryotic organism which includes microorganisms such as yeasts, moulds and mushrooms. Fungi perform an essential role in the decomposition of organic matter and have fundamental roles in the nutrient cycling and exchange. Since 1940's fungi was used for the production of antibiotics, and most recently, various enzymes produced by fungi which are used industrially and in detergents. Fungi can breakdown the manufactured materials and buildings. Losses of crops due to fungal diseases (e.g. rice blast disease) and food spoilage can have a large impact on human food supplies and local economics.

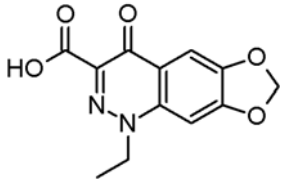
Antibiotic Resistance:

Antibiotic resistance is the type of drug resistance, where a microorganism is able to survive exposure to an antibiotic. Many antibiotics resistance genes reside of plasmids, facilitating their transfer. If a bacterium carries several resistant genes which is called multidrug resistant. Any use of antibiotics can increase selective pressure in population of bacteria to allow the resistant bacteria to thrive and the susceptible bacteria to die off. As resistance towards antibiotics becomes more common and a greater need for alternative arises.

Cinnoline ring is a versatile lead molecule[7] that has been investigated widely used in medicinal chemistry due to its important pharmacological activities. The nucleus gives out different derivatives with different biological activities[8-34]. This have been reported to exhibit anti-microbial, anti-tubercular, anti-malarial, anti-hypertensive, anti-convulsant, neurological disorders, anti-depressant, anti-pyretic, analgesic, anxiolytics, anti-diabetic, anaesthetic, anti-thrombolytic, cardiotonic, anti-tumor, herbicidal, agrochemical insecticidal, etc. In the present study, it is envisaged to combine Cinnoline nucleus with Pyrazoline ring system and Sulfonamide compounds have important biological activities.

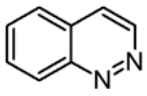
Cinnoline[35] is 1,2-diazonaphthalene the nitrogenous organic base, obtained from certain complex diazole compounds, it is benzfused Pyridazine containing two nitrogen atoms at 1,2 position and its categorized under benzfused diazines class of heterocyclic compounds. Its isosteric relative to either Quinoline or Isoquinoline. Some of the Cinnolines have been screened and have received approval as bioactive drugs are under clinical trials.

Cinoxacin (Cinobac) [98] is an, Quinolone, is 1-ethyl-1, 4-dihydro-4-oxo-[1, 3] dioxolo [4, 5-g] Cinnoline-3-carboxylic acid and occurs at white or very light yellow needle shaped crystals. It is a synthetic antibacterial agent for oral administration used in urinary tract infections. It is available as 250-500mg capsules.

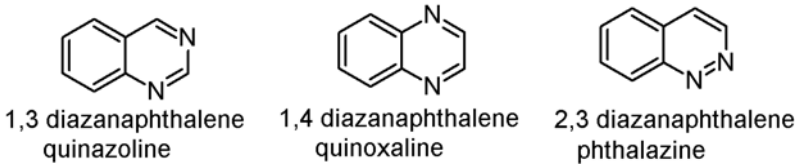


CHEMISTRY OF CINNOLINES

Cinnolines are coming under benzodiazines[36, 37]. Cinnoline is benzo analogs of Pyridiazine, Phthalazine benzo Pyridiazine, 1, 2 diazonaphthalene.

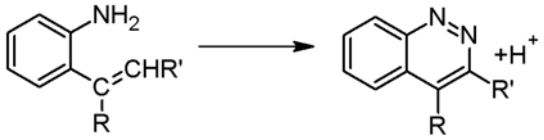


Other benzodiazines are,



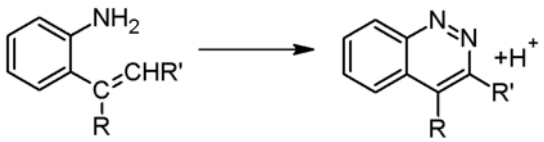
Methods of synthesis of Cinnolines:

A diazo group[38] attached to an aromatic ring can couple with an alkyl group in ortho position provided that the latter and is activated by an electronegative substituent. But since the activation does not extend beyond the α-carbon atom of the alkyl group coupling takes place at that position and a five-membered Pyrazole ring is formed. In general, Six-membered rings are formed more easily than five-membered ones. If an unsaturated chain of two or more carbon atoms takes place of the ortho-alkyl group, this chain is nucleophile at the ground state. And if the β-carbon atom acquiries a sufficient it may be substituted by an ortho diazonium ion so that a fused six membered ring containing two continuous nitrogen atoms are formed.



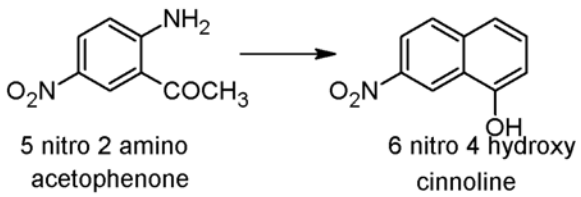
The substituents R & R' are mainly responsible for activation of the β-carbon atom. The product are the derivates of benzo-1, 2-diazine, also named benzopyridazine or Cinnoline. Conditions suitable for Cinnoline formation are present in the diazo compounds.

Widman[39] observed that a cold acid solution of diazotized 3-amino-4-propen-2-yl benzoic acid gives 4-methylCinnoline-7-carboxylic acid as a result of intramolecular coupling.



Borsche Reaction[40]:

This involves the diazotization of ortho-aminoacetophenones in aqueous medium which provides an easy access to Cinnolines.

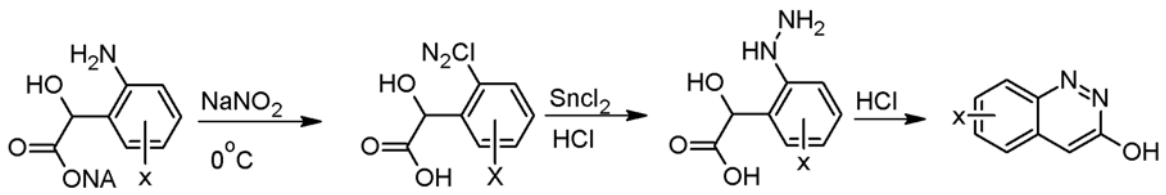


The Cinnoline ring was first synthesized by Richter during the diazotization of ortho-amino-Phenylpropionic acid and cyclization of the obtained arene diazonium salt.

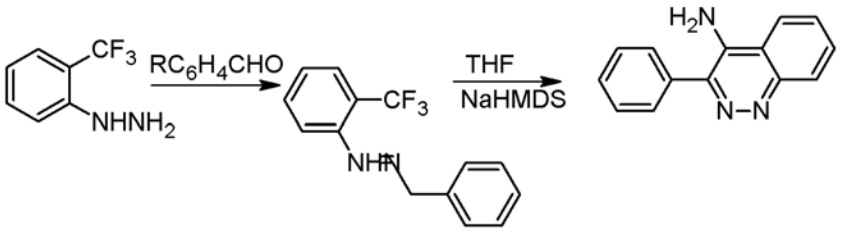
Three main approaches[41] for the synthesis of Cinnolines are using derivatives of arenediazonium salts, aryl hydrazones, and aryl hydrazines as precursors and also reductive methods for the synthesis of polycondensed derivatives of Cinnoline.

ARYLHYDRAZONES AND ARYLHYDRAZINES AS PRECURSOR OF CINNOLINES[42, 43]

This approach is the most universal since it makes it possible to obtain derivatives of Cinnoline with the various types of substituent at various positions which includes in the methods of the Cinnoline system is formed at various positions of Pyridazine ring. As a rule, ring closure occurs during attack of the amino group at a CC, CO, or CN multiple bonds. An example of the production of Cinnoline through the formation of the N (2)-C (3) bond is the classical method for the synthesis of 3-hydroxyCinnolines the Neber-Bossel method. During the diazotization of (2-aminophenyl) hydroxyacetate and reduction of the diazonium salt the obtained hydrazine undergoes cyclization to 3-hydroxyCinnolines. When boiled in HCL substituents in the aromatic ring have an appreciable effect on the course of cyclization, and in the case of the unsubstituted and 4-Chloro substituted ring the yields of the desired compounds are 60 and 70% respectively.



Hydrazones obtained from ortho-trifluoromethyl arylhydrazines[44] and benzaldehydes undergo cyclization by the action of a base, forming a Pyridazine ring. The products in this case are 4-amino 3-aryl Cinnolines, and their yields amount to 60-90%.



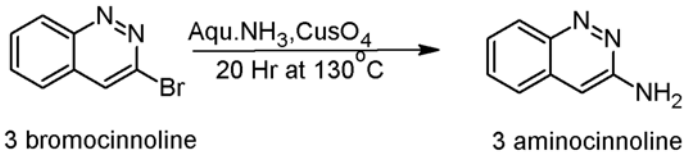
This approach was first described in 1956 by Barber and Co-workers. The formation of a Cinnoline ring with the participation of aryl hydrazones through the construction of a bond between the fourth carbon atom and the benzene ring can also be realized under the conditions of the Friedal-Crafts reaction[45]. Nowadays this method has been used for the synthesis of polycondensed derivatives of Cinnoline.

CHEMICAL REACTIONS

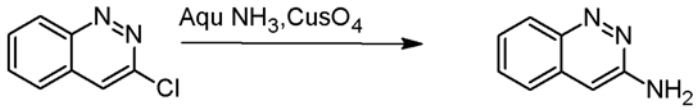
The chemical reactions of Cinnolines are,

Nucleophilic substitution reaction[46,47]:

Vigrous conditions are required for methoxylation of 3-Bromo, 3-ChloroCinnoline reacts with aqueous ammonia at 20 hr at 30^{o}C to form the amination of Cinnoline.



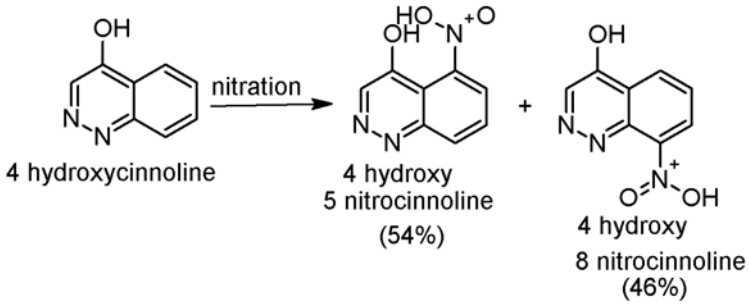
3 bromocinnoline 3 aminocinnoline



3 Chlorocinnoline 3 aminocinnoline

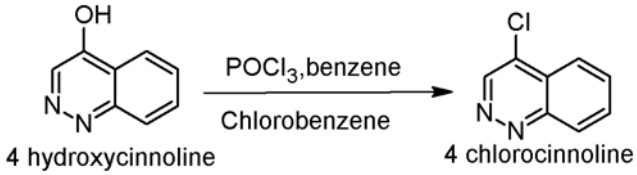
Electrophilic substitution reaction[48, 49, 50]:

The nitration of Cinnoline involves the conjugate acids formed by the addition of proton to the one of the nitrogen atom.



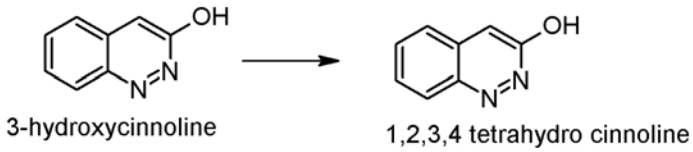
Halogenation[51, 52]:

4-HydroxyCinnoline is reacted with Phosphorous oxytrichloride and Pyridine to form 4-ChloroCinnoline.



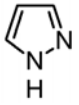
Reduction[53, 54, 55]:

The 3-HydroxyCinnoline reacts with the reduction of lithium hydride to form 1, 2, 3, 4-tetrahydro Cinnoline.



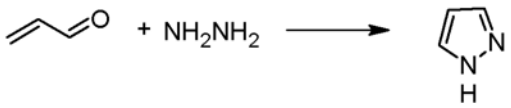
PYRAZOLE

Pyrazole[131] was given to this class of compounds by Ludwig knorr in 1883. It is the class of simple aromatic ring organic compounds of the heterocyclic diazole series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions, and to the unsubstituted parent compound.



Method of Pyrazole:

Pyrazoles are produced synthetically through the reaction of α, β unsaturated aldehydes with hydrazine and subsequent dehydrogenation.

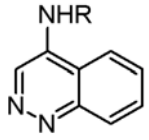


In medicine, derivatives of Pyrazoles are used for their analgesic, anti-inflammatory, anti-pyretic, anti-arrhythmic, tranquilizing, muscle relaxing, psychonaleptic, anti-convulsant, monoamineoxidase (MOA) inhibiting, anti-diabetic and anti-bacterial activities.

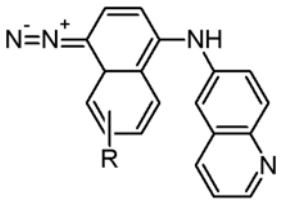
Literature Review

ANTI-MALARIAL

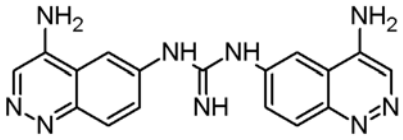
1. **Richer V. *et al*[83] With** the view of discovering new anti-malarial drugs such as Chloroquine analogs, the derivatives of 4-aminoCinnolines were synthesized **,** Biological tests demonstrated that some of them showed significant activity.



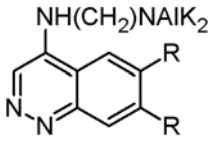
2. **Wieslawa Lawgowd *et al*[84]** Synthesized derivatives of Cinnoline diazanaphthalene derivatives and screened for anti-malarial activity**.**



3. **Lourie E.M *et al*[85]** synthesized Cinnoline compounds and screened for trypanosome congolense infection.

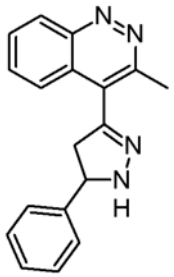


4. **Ken ford J.R *et al*[86]** synthesized a series of 6, 7 substituted 4-amino alkyl amino Cinnolines were prepared by the conversion of the 4-Hydroxy Cinnoline to 4-Phenoxy Cinnoline followed by condensation with amines, and their anti-malarial activity were recorded.

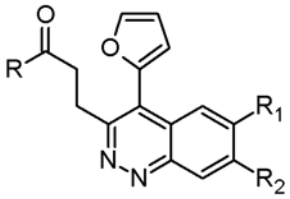


ANTI-MICROBIAL

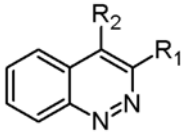
5. **Nidhi Gautam *et al*[87]** synthesized some novel Cinnoline based Chalcones and Cinnoline based Pyrazoline derivatives and screened for anti-microbial and insecticidal activity.



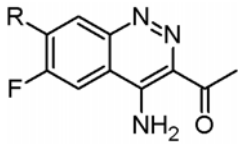
6**. Vladimir T. Abaev *et al*[88]** synthesized (Z)-4-[4- (5-Methyl-2-furyl)-3-Cinnolinyl]-3-buten-2-one and (Z)-1[4-(5-ethyl-2-furyl)-3-Cinnolinyl]-1-penten-3-ones have been obtained from 2-aminoarylbisfurylmethanes under treatment with isoamyl nitrite/trimethylchlorosilane in dry acetonitrile and potent antibacterial and anti-fungal activity observed.



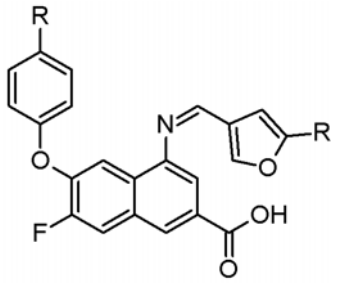
7**. Jacob T.L *et al*[89]** synthesized a new group of 3, 4-substituted Cinnolines and screened for antimicrobial activity and all the derivatives exerted bacteriostatic activity against Gram-positive bacteria and spasmolytic action.



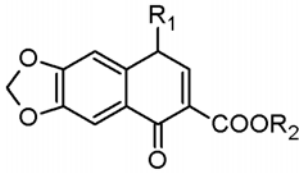
8**. Hipparagi S.M *et al*[90]** synthesized a series of 7-substituted 6-fluro, 4-aminoCinnoline -3-carboxamides and evaluated for antibacterial activity, several such compounds showed antibacterial activity.



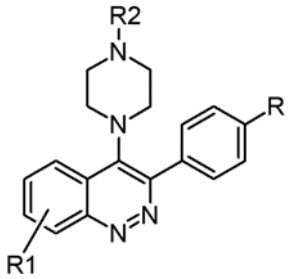
9. **Saravanan J. *et al*[91]** synthesized eight new 7-substituted Cinnoline -3 -carboxylic acids and screened for anti-microbial activity. some of the compounds exhibited comparable antimicrobial activity with standard drugs at same concentration and 4[5-Substituted-2-Furanyl) Amino]-7-Substituted Aryloxy-6-Fluoro Cinnoline-3-Carboxylic Acids was found to be most potent.



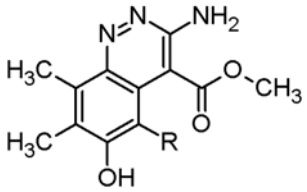
10. **Conrad *et al*[92]** synthesized series of 1-alkyl-1, 4-dihydro-4oxo [1, 3] dioxolo[g] Cinnoline-3-carboxylic acid, and tested for their antibacterial profile against gram-negative bacteria. Some of them, e.g., 1-ethyl-1, 4-dihydro-4-oxo [1, 3] dioxolo[g] cinnoline-3-carboxylic acid.



11. **Lowrie H.S. *et al*[93]** shown 3-phenly-4-piperazinyl Cinnolines has Anti-bacterial activity and diuretic activity.

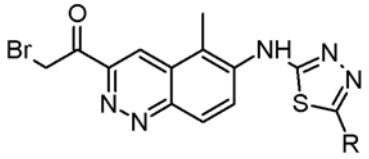


12. **Chung-Kyu Ryu, Jung yoon Lee[94]** Synthesized 6-HydroxyCinnolines & cyclohexa 2, 5-diene-1, 4-dione derivatives and screened for *invitro* antifungal activity against *candida* species & *Aspergillus niger.* Among them, 2-amono-7, 8-dimethyl-6-hydroxy Cinnolines exhibited potent antifungal activity.

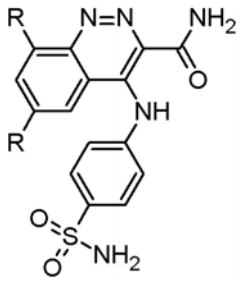


13. **Anshu jakhar et al[95]** synthesized a series of 2-substituted 6-(4-methyl-6-substituted Cinnoline-3yl) imidazo(2,1-b) [1,3,4] thiadiazole by reacting 3-(2-bromoacetyl)-4-methyl-6-substituted Cinnoline with

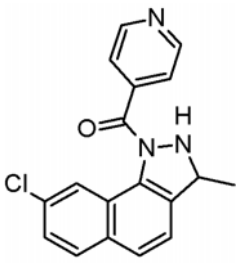
various 2-amino-5-substituted-1,3,4 thiadiazoles and tested for their anti-bacterial properties against gram positive and gram negative bacteria**.**



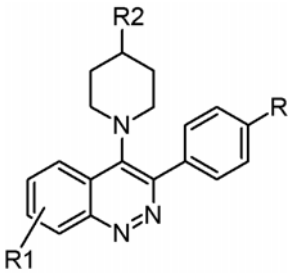
14**. Vikas *et al*[96]** Synthesized 6 and 8 substituted 4-(p-amino phenyl- sulphonamide) Cinnoline 3-carboxamide with good antimicrobial properties.



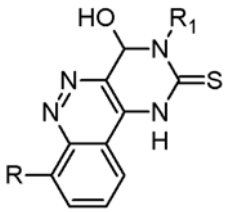
15. **Rajiv Kumar *et al*[97]** synthesized **(**8-Chloro-3-methyl-1H-Pyrazolo Cinnolin-1-yl) (Pyridin-4-yl) methanone through condensation of 3-acetyl-6-chloro-1*H*-Cinnolin-4-one with isonicotinic acid hydrazide (INH) in absolute ethanol and potent antibacterial and antifungal activity observed.



16. Cinoxacin (Cinobac, noxigram) as analogs of oxolinic acid was used in urinary tract infections. **Miyamato[98]** shows Cinoxacin is active against *Mycoplasma gallisepticum, Escherichia coli*, and *salmonella Dublin, vibrio coli* and *Xanthomonas phaseali and* also the anxiolytic activity.

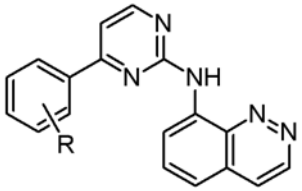


17**. Narqud *et al*[99]** synthesized derivatives of 2-mercapto-3-arylpyrimido [5, 4-c] Cinnolin-4 (3H)-ones some of them showed potent anti-microbial and antifungal activities.

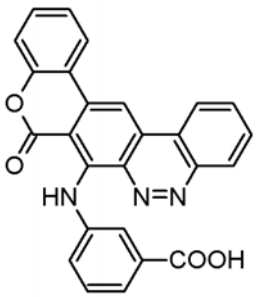


ANTI-TUBERCULAR

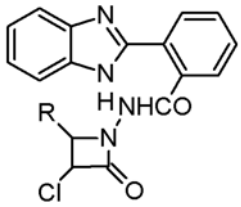
18. **Ravindra Reddy *et al*[100]** synthesized some substituted Pyrimido Cinnolines and screened for anti-tubercular activity against *Mycobacterium tuberculosis* and some compounds have shown anti-tubercular activity.



19. **Ramalingam P. *et al*[101]** reported synthesis of Coumarino [4, 3-b] Pyrido [6,5-c] Cinnolines as potent anti-tubercular agents.

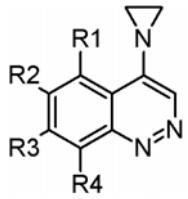


20. **Preethi, Kagthara *et al*[102]** reported synthesis of some 2-azetidinones as potent anti-tubercular agents.

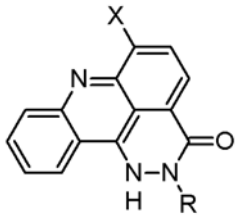


ANTI-TUMOR

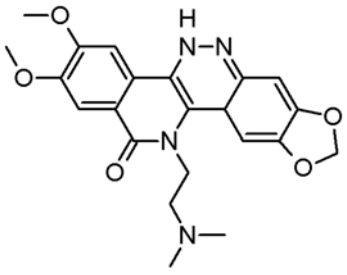
21. **Yamazaki T. *et al*[103]** prepared a series 4-aziridino Cinnolines, exhibited antitumor activity with substance at 5,6,7,8 positions.



22. **Edward Borowski *et al*[104],** were synthesized a series of Pyridazinoacridin-3-one derivatives & evaluated for their cytotoxic activity towards murine & human leukemia sensitive and resistant (MDR & MRP) cell lines.

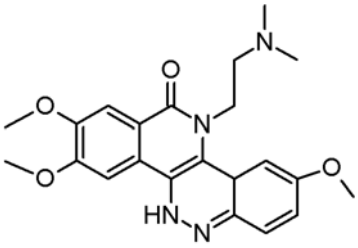


23.**Alexander *et al*[105]** Studies have identified 2, 3-dimethoxy-8, 9-methylenedioxy-11-[(2-dimethylaminoethyl]-11H-isoquino[4,3-c]Cinnolin-12-one as a novel topoisomerase I-targeting agent with potent cytotoxic activity. The effect of varied substituents at the 11-position of 2, 3-dimethoxy-8, 9-methylenedioxy-11H-isoquino [4, 3-c] Cinnolin-12-ones on topoisomerase I-targeting activity and cytotoxicity was evaluated. Potent TOP1-targeting activity was observed when the 11-position was substituted.

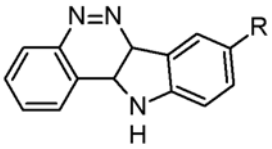


24. **Wei Feng *et al*[106]** observed Facile formation of hydrophilic derivatives of 5H-8, 9-Dimethoxy-5-[2-(N, N-Dimethylaminomethylenedioxydibenzo[c, h][1,6]naphthyridin-6-one (ARC-111) and its 12-aza analog via

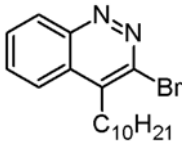
quaternary ammonium intermediates have proven to be a particularly promising family of non-camptothecin TOP1-targeting agents.



25**. Anna maria almerico *et al*[107],** prepared a series of Indolo [3,2-c] Cinnoline derivatives and tested to evaluate their biological activity, most of them inhibited the proliferation of leukemia , lymphoma and solid tumor derived cell line, and all compound show activity against gram positive bacteria.

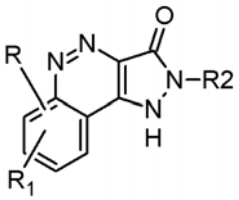


26**. Viktor N. Sorokoumov *et al*[108]** studied a short route to 3-alkynyl-4-bromo (chloro) Cinnolines by Richter-type Cyclization of ortho-(dodeca-1, 3-diynyl) aryltriaz-1-enes, and exhibited potent antitumor activity**.**

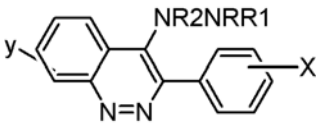


ANTIHYPERTENSIVE AND ANTIPSYCHOTIC

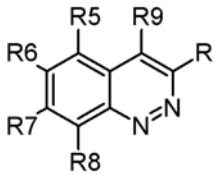
27**. Schenker E. *et al*[109]** Synthesized Pyrazolo Cinnoline as psychotropic agents and observed 3-amino-tetrahydro Cinnoline derivatives possess hypotensive properties.



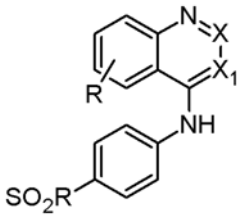
28. **Lowrie *et al*[110]** synthesized .4-amino-3-PhenylCinnolines it exerted antihypertensive, antibacterial, and fungicidal activity.



29**. Jpn.Kokai Tokyo Koho *et al*[111]** synthesized Cinnoline-3-carboxamides which exerted sedatives anxiolytic activity, tranquilizers.

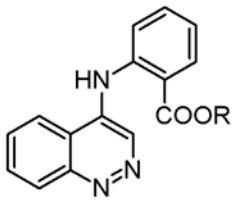


30. **Boyle *et al*[112]** synthesized new analogs of Sulfonamides 4-aminocinnoline these compounds exerted hypotensive properties.

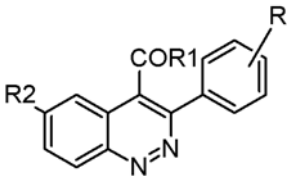


ANTI-INFLAMATORY AND ANTI- ALLERGIC

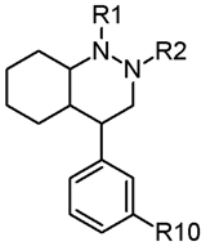
31**. Allais *et al*[113]** synthesized 4-aminoCinnoline derivatives and showed anti-inflammatory properties and also analgesic and antiphlogistic activity.



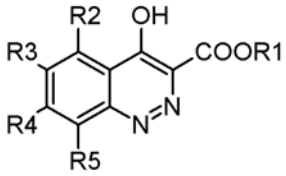
32**. Lowrie *et al*[114]** synthesized 3-PhenylCinnoline-4-carboxylic acid derivatives and the amides, amino alkyl amides and hydrazides derivatives showed anti-inflammatory properties.



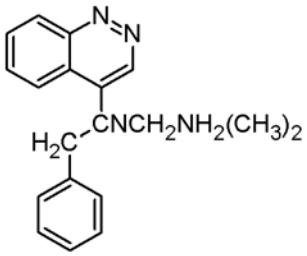
33**. Kikazawa K. *et al*[115]** synthesized derivatives of decahydro Cinnolines with strong analgesic action**.**



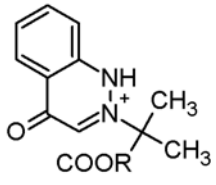
34. **Gilman's patent *et al*[116]** provided various 4-hydroxy Cinnoline-3-carboxylic acids and related esters as potentially anti-allergic drugs for Asthma.



35**. Nunn A.J. *et al*[117]** synthesized Series of ethylenediamine derivatives with Cinnoline moiety showed moderate anti-histaminic activity.



36. **Dron H. *et al*[118]** synthesized 4-OxoCinnoline derivatives, all the derivatives showed anti-inflammatory and analgesic properties, and Lowered high cholesterol levels.



Aim & Objective

AIM OF THE PRESENT WORK

Malaria[56] is the most dreadful illness and widespread infectious disease because of its prevalence, virulence and drug resistance, having an overwhelming impact on public health in developing regions of the world. It affects more than 2400 million people, over 40% of the world population. Casual organism for malaria belongs to genus *Plasmodium*. *Plasmodium falciparum* is the main cause of severe clinical malaria and the World Health Organisation (WHO) has forecast an annual growth of 16% in malarial cases. As the parasites rapidly develop permanent resistance against the different subclasses of existing drugs, there is a great urge to develop new and effective drugs attacking crucial targets in the metabolism of the malarial pathogen. Malaria can be eliminated by proper drug therapy.

Mycobacterium tuberculosis[62], the causative agent of tuberculosis (TB), is a tenacious and remarkably successful pathogen that has latently infected a one-third of the world population. It can be cured, the therapy takes at least 6-9 months and laborious and lengthy treatment brings with it dangers of non-compliance, significant toxicity and drug resistance. The increasing emergence of drug resistance and the problem of mycobacterial persistence, prolonged therapy, and drug toxicity highlight the need to develop novel TB drugs that are active against drug resistant bacteria.

Microorganisms[78, 79] like bacteria and fungi causes many infections like meningitis**,** ottis media, pneumonia, cholera, food poisoning, urinary tract infections, aspergillosis, candidoses, etc. Many of these diseases are fatal if untreated, and treatment has been complicated by the resistance of the microorganisms to the widely used drugs. To combat the problem of resistance newer drugs are needed.

The increasing emergence of drug resistance highlights the need to develop some novel active Anti-malarial, Anti-tubercular and Anti-microbial drugs.

Cinnoline[35] is, 1, 2-diazanaphtalene or benzo[c]-1, 2-diazine (Hantsch-Widmann system), it is a nitrogenous organic base, obtained from certain complex diazole compounds. It is a benzfused pyridazine containing two nitrogen atoms at 1, 2-position and it is categorized under benzfused diazines class of heterocyclic compounds. Its isosteric relative to either quinoline or isoquinoline.

Cinnoline ring is a versatile lead molecule[7] that has been investigated widely in medicinal chemistry due to its important pharmacological activities[8-34]. It have been reported to exhibit anti-microbial, anti-

tubercular ,anti-cancer, anti-malarial, anti-hypertensive, anti-pyretic, anti-thrombolytic, analgesic, anti-diabetic, anti-depressant, cardiotonic, anaesthetic, anxiolytic etc.

In medicine[131], derivatives of Pyrazoles are used for their analgesic, anti-inflammatory, anti-pyretic, anti-arrhythmic, tranquilizing, muscle relaxing, anti fungal, anti tubercular, psychonaleptic, anti-convulsant, monoamineoxidase (MOA) inhibiting, anti-diabetic and anti-bacterial activities.

The Anti-malarial, Anti-tubercular, and Anti-microbial activity of Cinnolines and the well known biological importance of Sulfonamides, Pyrazole prompted us to substitute these into Cinnoline nucleus, hoping to get compounds with enhanced potency against Plasmodium protozoa, Mycobacterium, bacteria and fungus which may overcome the existing lacuna in the current treatment of these infectious diseases.

The objectives of the present work can be summarized as follows:

- Synthesis of some Novel potential derivatives of Cinnoline fused with Sulfonamide and Pyrazoline group.
- Characterization of synthesized compounds by various analytical techniques like TLC, UV, IR NMR and Mass Spectral studies**.**
- Screening for anti-malarial activity against *Plasmodium falciparum* by culturing *Plasmodium faliciparum* from infected human blood sample by using Candle Jar method.
- Screening for anti-tubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ by Resazurin Dye Reduction Assay method.
- Screening for anti- bacterial activity against bacterial organisms like *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Corynebacterium diphtheria, Bacillus linctus, Escherchia coli, Pseudomonas aureginosa, Rhodosporum rubrum, Vibrio cholera* and *Salmonella paratyphi* by Disc diffusion method and determination of Minimum inhibitory concentration (MIC) by Serial dilution method.
- Screening for anti-fungal activity against fungal organisms like *Candida albicans, Streptomyces griseus, Aspergillus niger, Aspergillus fumigatus, Monascus ruber,* by Disc diffusion method and determination of Minimum inhibitory concentration (MIC) by Serial dilution methods.

Experimental Work

SYNTHESIS OF SOME NOVEL CINNOLINE DERIVATIVES[87]

METHODOLOGY

STEP1

Preparation of diazonium salt [Diazotisation]

Sodium nitrite (7.4gm,0.1mol) dissolved in 26ml of water was added to a suspension of Sulfanilamide (10gm,0.1mol) in 1N HCl (200 ml), and the mixture was stirred for 1hr at 0-5˚C and filtered to obtain the clear diazonium salt.

STEP 2

Preparation of Phenyl hydrazano acetyl acetone-4-Sulfonamide [Griess diazo reaction]

The diazonium salt obtained was then added to a well stirred solution of ethanol (30ml), water (500ml) and acetyl acetone (10.01gm, 0.1mol) at 0ºC with stirring. Sodim acetate was then added to keep the mixture alkaline to litmus after 3 hour stirring at 0ºC the crude product was filtered, washed with water and air dried. Recrystallisation from ethanol afforded yellow needles of purified Phenyl hydrazano acetyl acetone-4-Sulfonamide.

The purity of product obtained was established by single spot on the TLC plate. The solvent system used was Benzene: Ethyl acetate (8: 2). Melting point was determined and uncorrected.

STEP 3

Preparation of 4-methyl 3-acetyl Cinnoline 6-Sulfonamide [Intramolecular cyclization]

The Phenyl hydrazano acetyl acetone-4-Sulfonamide (10g, 0.05mol) was added to the Polyphosphoric acid (16gm, 7.216 ml, 0.03mol) in small lots over 30 mins while maintaining the temperature between 60-65^{0}C. The reaction was maintained for an additional 2 hour and monitored by TLC. After the completion of reaction, icecold water (200 ml) was added carefully to decompose the black residue at 0-5^{0}C. The product was then extracted with ethyl acetate. Ethyl acetate layer was then treated with Charcoal and concentrated to get the crude product as a brownish black residue. Recrystallisation from methanol to obtained as light yellow crystals of 4-methyl 3-acetyl Cinnoline 6-Sulfonamide.

The purity of the product was confirmed by a single spot on the TLC plate and solvent system used was Benzene: Ethyl acetate (8:2). Melting point was determined and uncorrected.

STEP 4

Preparation of 1-(4-methyl Cinnoline-3-yl)-3-(substituted phenyl) prop-2-en-1-one 6-Sulfonamide [Cinnoline based chalcone]

The product obtained from step 3(2.03gm,0.01mol) and aromatic aldehyde in same ratio (0.01mol) in ethanol (50ml) was cooled at 0-5^{o}C and added (5-10ml) 40% NaOH solution till precipitated and washed with ice water. Few drops N/20 dilute HCl was added for complete precipitation and filtered washed with ice water and recystallised from alcohol to afford the compound (CN-1 -11).

The purity of the product was confirmed by a single spot on the TLC plate and solvent system used was Benzene: Ethyl acetate (8:2). Melting point was determined and uncorrected.

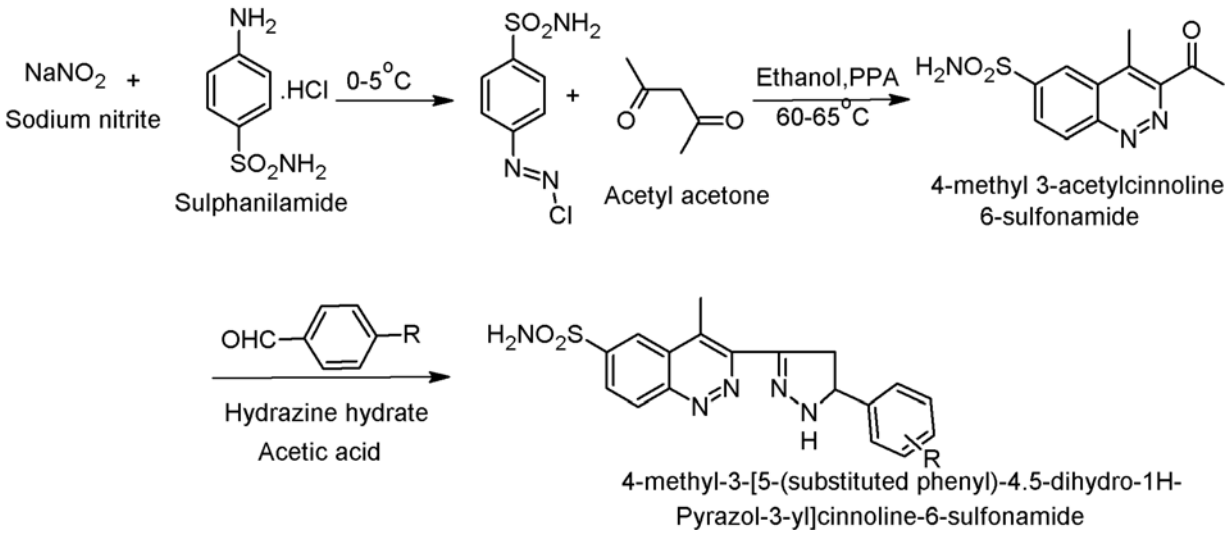
STEP 5

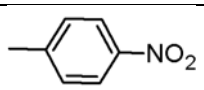
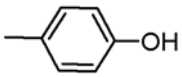
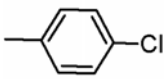
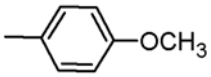
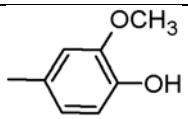
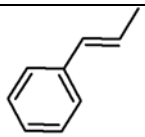
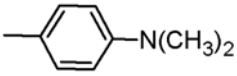
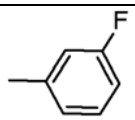
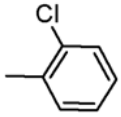
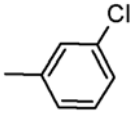
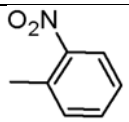
Preparation of 4-methyl-3-[5-(substituted phenyl)-4, 5-dihydro-1H-Pyrazol-3-yl] Cinnoline-6-Sulfonamide [Intermolecular cyclization]

The compound CN-1-11 (0.01mol) in 20ml acetic acid was taken and hydrazine hydrate (0.01mol) was added to it and refluxed for 10 hour. The contents were poured into ice, filtered and the product isolated, crystallized from ethanol to afford the compound (CN-1a-11a).

The purity of the product was confirmed by a single spot on the TLC plate and solvent system used was Benzene: Ethyl acetate (8:2). Melting point was determined and uncorrected.

SCHEME


NaNO2 +
Sodium nitrite
NH2
.HCl
0-5°C
SO2NH2
Sulphanilamide
SO2NH2
N=N
Cl
Acetyl acetone
Ethanol,PPA
60-65°C
H2NO2S
O
N=N
4-methyl 3-acetylcinnoline
6-sulfonamide
OHC
R
Hydrazine hydrate
Acetic acid
H2NO2S
N=N
N
N
H
R
4-methyl-3-[5-(substituted phenyl)-4.5-dihydro-1H-
Pyrazol-3-yl]cinnoline-6-sulfonamide

S.NO	Compound code	R
1	CN-1a	
2	CN-2a	
3	CN-3a	
4	CN-4a	
5	CN-5a	
6	CN-6a	
7	CN-7a	
8	CN-8a	
9	CN-9a	
10	CN-10a	
11	CN-11a	

Biological Screening

ANTI MALARIAL SCREENING

CANDLE JAR METHOD[121, 122,123]

PROCEDURE

Preparation of Serum:

- Fresh human whole blood (preferably o+ or A+) was collected in a blood collection bags without anticoagulant. And (before clotting occurs) the blood was transferred to the sterile 50ml centrifuge tubes. (In a laminar flow cabinet)
- The blood was stored overnight at 4^oC to allow clotting.
- The serum and erythrocytes was fractionated by centrifugation. (2500 rpm for 30mins at room temperature)
- The serum was then transferred under sterile conditions to new sterile 50ml tubes.
- The serum was then heat inactivated by incubation in a water bath for 20mins at 65^oC.
- The serum was then stored at -20^oC in the 50ml sterile tubes.

P.falciparum culture: Media and Buffers (Serum):

The medium used for culturing of *Plasmodium falciparum* was prepared as follows:

Ingredients used

S.NO	INGREDIENTS	QUANTITY
1	RPMI-1640(sigma)	10.4g
2	HEPES (sigma, cell culture tested)	5.94g
3	D-glucose (sigma, cell culture tested)	4.0g
4	Hypoxanthine(sigma, cell culture tested)	44mg
5	Gentamycin	0.4ml
6	Distilled water	1000ml

The accurately weighed quantities of the above ingredients were transferred into the conical flask, mixed well and leave for 30 minute at room temperature. Add 36ml of 5% $NaHCO_3$ solution was added to the medium to buffer the solution at pH of 7.4. The solution was filtered using a sterile Millipore filter (0.22mm). Filtered solution was divided into two halfs. Transferred half to the 500ml medium bottle and stored at 4^oC (wash medium). 50ml human serum was added to the other half and transferred to a 500ml medium bottle and stored at 4^0C(culture medium).

Preparation of Erythrocytes:

- Erythrocytes were collected in a 10ml vacutainer (vacutest) tubes with anticoagulant (EDTA purple).
- The bloods were transferred under sterile conditions to the sterile 10ml centrifuge tube.
- It was centrifuged to separate the cells and serum (2500 rpm for 5mins at room temperature).
- The serum and "Buffy-coat" was aspirated using a Pasteur pipette fixed to the vacuum line.
- The volume was then supplemented to 10ml with "wash medium" and mixed well.
- The cells were centrifuged (2500 rpm for 5mins at room temperature).
- Steps 4 to 6 were repeated another two times.
- The washed erythrocytes was resuspended in wash medium and stored at $4^{o}C$.

Cultivation of Parasites:

Preheat the culture medium by placing it in the $37^{o}C$ water bath or in the incubator. O.5ml of culture medium, 20µl of washed erythrocytes and 20µl of *P.falciparum* infected human blood were placed in 96 well plates. The well plates were placed in a candle jar and incubated at $37^{o}C$ for 24hrs. Parasite counts were made on giemsa stained thin smears.

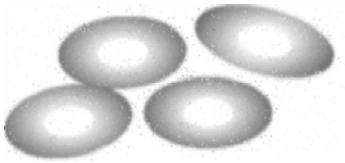
Preparation of thin smears:

- A small amount of erythrocytes were removed using a Pasteur pipette and placed on a microscopic slide. The second slide was placed on the first and moves it back into the drop, spreading it. The second slide was then moved forward, smearing a film of blood across the first slide.
- The smear was allowed to dry and then it was fixed with methanol.
- The methanol was discarded and the slide was covered with giemsa stained solution for 3 to 5 minutes to stain. (The giemsa solution consists of approximately 0.5ml phosphate buffer and 10-20 drops of concentrated giemsa stock solution). Then rinsed with water and allowed it to dry, where after it was studied microscopically (100x).
- Parasitemia were counted by counting both uninfected and parasite infected erythrocytes.
- Expressing the parasite count as a percentage of infected erythrocytes as per total erythrocyte count.

Interpretation of microscopic slides:

Different stages of malaria parasite in the red blood cells interpreted.

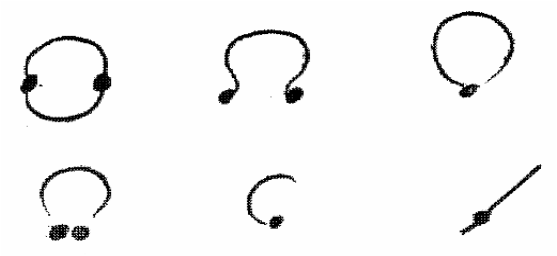
Uninfected erythrocytes



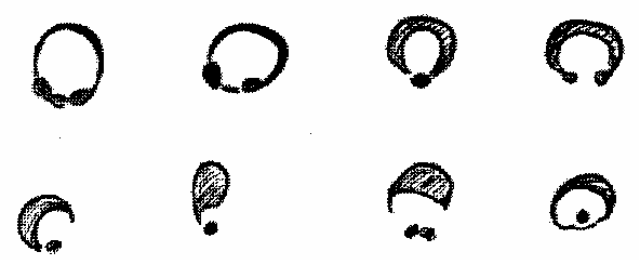
Infected erythrocytes

Different stages of the malarial parasite in the red blood cells.

Ring:

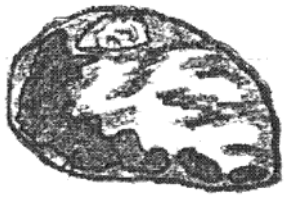


Early Trophozoite:



Trophozoite:

Transition Stage:



Anti-Plasmodial activity of the synthesized compounds:

Various concentrations of the synthesized compounds and standard drug pyremethamine (20,40,60,80 µg/ml) in DMSO, was added to the well plates containing 0.5ml of culture medium, 20 µl of washed erythrocytes and 20 µl of *P.falciparum* infected human blood. Well plates were placed in the candle jar and incubated at 37^{o}C for 24hrs. Parasite count were made on the giemsa stained thin smears prepared at different time intervals (24, 48, 72 hrs). And *in-vitro* anti-plasmodial activity of the synthesized compounds was determined by calculating inhibitory concentration percentage (IC_{50}). Percentage can be calculated using the formula,

$$\mathbf{IC_{50}} = \frac{\text{No of parasitemia in control} - \text{No of parasitemia in treated}}{\text{No of parasitemia in control}} \times 100$$

ANTI-TUBERCULAR SCREENING[124]

RESAZURIN DYE REDUCTION ASSAY

Resazurin is an oxidation reduction indicator used for the evaluation of cell growth, particularly in various cytotoxicity assays. It is a blue non fluorescent and non toxic dye that becomes pink and fluorescent when reduced to resorufin by oxide reductive within viable cells.

PROCEDURE:

Mycobacterium tuberculosis $H_{37}Rv$ was grown in Middle brook 7H9 broth (Difco, USA) containing 0.05% Tween 80, 0.05% Glycerol and 5% OADC supplement (Becton Dickinson, USA). The culture was grown till log-phase and was diluted to McFarland 1 standard with the same medium. From these 50 micro litres was added to 450 micro litre of fresh medium in 2ml eppendorf tubes. Stock solution of the test compounds was prepared in DMF/DMSO. And the compounds were tested at 1, 10 & 100 micro gram per ml concentrations. And Rifampacin 1µg/ml was used as positive control, control tubes had the same volume of DMF/DMSO without any compound. After incubation at 37^{o}C for 7 days, 30 microlitre of 0.01% resazurin (Sigma, St. Louis. MO. USA) in water was added to each well. Resazurin, a redox dye, is blue in the oxidized state and turns pink when reduced by growth of viable cells. The control tubes showed change of colour from blue to pink after 24 hours at 37^{o}C. Compounds in the well which remained blue were considered to inhibitory to *M.tuberculosis* at their respective concentrations.

ANTI-BACTERIAL SCREENING

DISC DIFFUSION METHOD[125, 126,127]

PROCEDURE

Preparation of Muller Hinton Agar Medium:

Composition of Muller Hinton Agar Medium

S.NO	INGREDIENTS	QUANTITY
1	Beef extract	10g
2	Casein acid hydrosylate	17.5g
3	Starch	1.5g
4	Agar	20g
5	Distilled water	1000ml

Specified amount of Muller Hinton Agar was taken along with 1000ml of distilled water in a conical flask and then heated in a steam bath to dissolve. The pH was maintained at 7.6 ± 0.2 and sterilized in an autoclave at 15 lb pressure, 120^{o}C for 15 minutes. The sterile medium was poured into the sterile Petri dish and allowed to solidify.

Preparation of plates:

Muller Hinton agar medium were prepared and transferred into the sterile Petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temperature. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on the levelled surface. Standardized bacterial inoculums of *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Corynebacterium diphtheria, Bacillus linctus, Escherichia coli, Pseudomonas aureginosa, Rhodosporum rubrum, Vibrio cholera, Salmonella paratyphi* were applied to the plates and spreaded uniformly over the surface of the medium by using a sterile non- absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs (100µg/disc) in dimethyl sulphoxide and standard ciprofloxacin 100µg disc were placed on the inoculated agar medium. All petriplates were incubated at 37^{o}C for 24 hrs. After the incubation produced by the sample were measured. The anti-bacterial activity were evaluated by measuring zone if inhibition in mm.

Determination of Minimum Inhibitory Concentration of Synthesized compounds (MIC) by Serial dilution Method[128]:

- The serial dilution of known concentration of the compound solution was made from the stock (10 µg/ ml) by using Muller Hinton broth using the method described below.
- The test tubes were labelled 1 to 8 and 1ml of Muller Hinton broth were added to the first 5 tubes and 8^{th} tube, then added 0.5ml Muller Hinton broth to 6^{th} and 7^{th} tubes.
- One ml of different synthesized compounds was added to the 1^{st} tube, mixed well and transfer 1ml serially upto tube 5. From the 5^{th} tube transfer 1ml to 6^{th} tube. Mixed and transfer 0.5ml to the 7^{th} tube, 1 to 7 contains 1ml diluted synthesized compounds.
- The 8^{th} tube was the control.
- With a standardized micro pipette, add a drop of the diluted broth culture approximately 0.01ml of the test organism to all test tubes, including the control, gently mixed and incubated at $37^{o}C$ for 24hrs.
- The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC.

ANTI-FUNGAL SCREENING

DISC DIFFUSION METHOD[129, 130]

PROCEDURE

Preparation of Sabourands dextrose broth:

Composition of Sabourands dextrose broth

S.NO	INGREDIENTS	QUANTITY
1	Dextrose	40g
2	Peptone	10g
3	Water	1000ml

Specified amount of dextrose and peptone was taken along with 1000ml of distilled water in a conical flask and heated in a steam bath to dissolve. The pH was maintained at 7.6 ± 0.2 and sterilized in an autoclave at 15 lb pressure, 120^{o}C for 15 minutes. The sterile medium was poured into the sterile Petri dish and allowed to solidify.

Preparation of plates:

Sabourands dextrose broth medium were prepared and transferred into the sterile Petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium was uniform in thickness, is done by placing the plates on a levelled surface. Standardized fungal inoculums of *Candida albicans, Streptomyces griseus, Aspergillus niger, Aspergillus fumigatus, Monascus ruber* were applied to the plates and spreaded uniformly over the surface of the medium by using a sterile nson-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs (100µg/disc) in dimethyl sulphoxide and standard Clotrimazole 100µg/disc were placed on the inoculated agar medium. All petriplates were incubated at 27^{o}C – 28^{o}C for 48 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured.

Determination of Minimum Inhibitory Concentration of the Synthesized Compounds (MIC) by serial dilution Method:

- The serial dilution of known concentration of compound solution was from the stock (10 µg/ml) by using Sabourands dextrose broth using the method described below.
- The test tubes were labelled 1 to 8 and 1ml of Sabourands dextrose broth were added to the first 5 tubes and 8^{th} tube, then added 0.5ml of Sabourands dextrose broth to 6^{th} and 7^{th} tube.
- One ml of different synthesized compounds was added to the 1^{st} tube, mixed well and transfer 1ml serially upto tube 5. From the 5^{th} tube transfer 1ml to 6^{th} tube. Mixed and transfer o.5ml to the 7^{th} tube. Each tube, 1 to 7 contains 1ml diluted synthesized compounds.
- The 8^{th} tube was the control.
- With a standardized micro pipette, add a drop of the diluted broth culture approximately 0.01ml of the test organism to all test tubes, including the control, gently mixed and incubated at 26-28^{o}C for 48 hrs.
- The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC.

Results and Discussion

RESULTS AND DISCUSSION

Table 1 .PHYSICO CHEMICAL PARAMETERS OF SYNTHESIZED DERIVATIVES

Sl. No	Compound code	Molecular formula	Molecular weight (g)	Percentage yield (%)	Colour	Solubility	Melting Point (^{0}C)	R_f value *
1	CN-I	$C_{11}H_{11}N_3O_3S$	265.29	76%	Brownish yellow	DMSO	85^{o}C	0.42
2	CN-1a	$C_{18}H_{16}N_6O_4S$	412.42	92.5%	Yellow	DMSO	82^{o}C	0.68
3	CN-2a	$C_{18}H_{17}N_5O_3S$	388.42	86.1%	Yellow	DMSO	110^{o}C	0.72
4	CN-3a	$C_{18}H_{16}N_5O_2S$	366.42	87.2%	Yellow	DMSO	85^{o}C	0.71
5	CN-4a	$C_{19}H_{19}N_5O_3S$	397.45	90.7%	Yellow	DMSO	87^{o}C	0.63
6	CN-5a	$C_{19}H_{19}N_5O_4S$	413.45	92.2%	Yellow	DMSO	82^{o}C	0.52
7	CN-6a	$C_{20}H_{19}N_5O_2S$	393.46	86.1%	Golden brown	DMSO	80^{o}C	0.67
8	CN-7a	$C_{20}H_{22}N_6O_2S$	410.49	91.4%	Yellow	DMSO	210^{o}C	0.75
9	CN-8a	$C_{18}H_{16}FN_5O_2S$	385.42	76.2%	Yellow	DMSO	92^{o}C	0.62
10	CN-9a	$C_{18}H_{16}ClN_5O_2S$	401.87	82.4%	Yellow	DMSO	85^{o}C	0.66
11	CN-10a	$C_{18}H_{16}ClN_5O_2S$	401.87	74.2%	Brownish yellow	DMSO	87^{o}C	0.56
12	CN-11a	$C_{18}H_{16}N_6O_4S$	412.42	89.5%	Yellow	DMSO	112^{o}C	0.58

*Mobile phase – benzene: ethyl acetate (8:2)

ANTI-MALARIAL SCREENING

CANDLE JAR METHOD

Table 3: *In vitro* anti Plasmodial activity of synthesized compounds against *Plasmodium falciparum*.

S.No	Compound Code	IC_{50} Values (%)			
		20 (µg/ml)	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**
1	Pyremethamine	72.30	80.15	87.69	95.32
2	CN-1a	59.23	66.92	74.61	82.30
3	CN-2a	46.15	51.53	66.92	72.30
4	CN-3a	36.15	46.15	53.84	61.53
5	CN-4a	49.23	56.92	64.61	72.30
6	CN-5a	69.23	76.92	84.61	87.69
7	CN-6a	30.76	38.46	43.84	50.00
8	CN-7a	41.53	46.15	57.69	61.53
9	CN-8a	52.30	61.53	69.23	76.92
10	CN-9a	27.69	42.30	51.53	56.92
11	CN-10a	33.84	43.84	61.53	72.30
12	CN-11a	61.53	69.23	76.92	84.61

ANTI-TUBERCULAR SCREENING

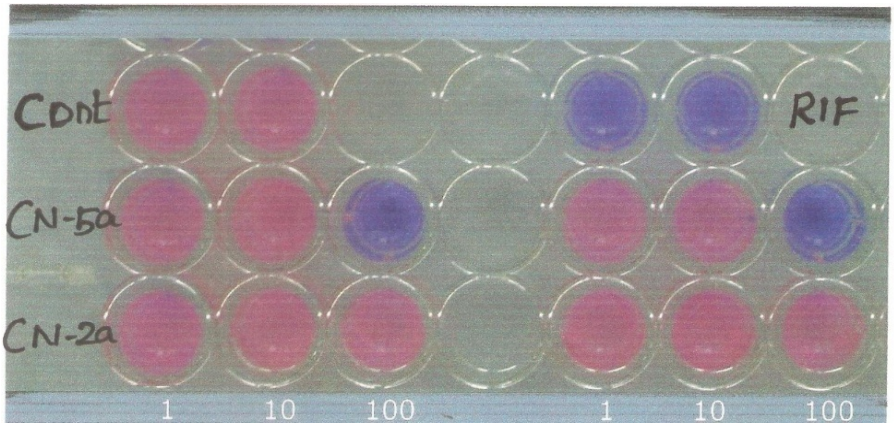
RESAZURIN DYE REDUCTION ASSAY

Table 4**:** *In vitro* Anti -mycobacterial activity of the synthesized compounds against *Mycobacterium tuberculosis* $H_{37}R_V$.

S.No	Compound code	Concentration of synthesized compounds		
		1µg/ml	10µg/ml	100µg/ml
1	CN-5a	N	N	P
2	CN-2a	N	N	N
3	RIF	P	P	P

P = Inhibition

N = No Inhibition



Control - Drug free control

Rifampicin 1µg/ml – Positive drug control

ANTI-BACTERIAL SCREENING

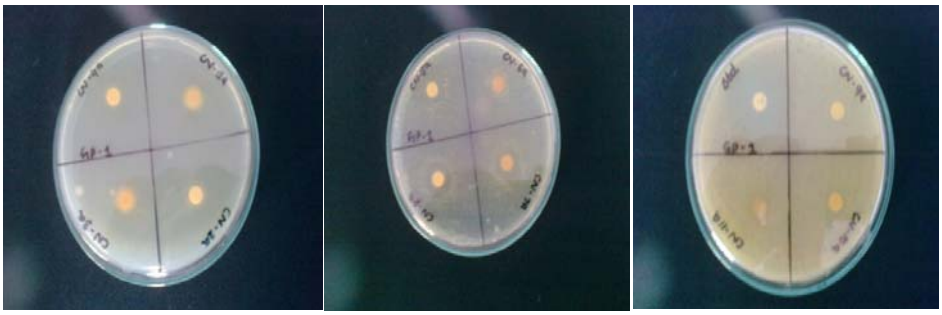
DISC DIFFUSION METHOD

Table 5: Anti-bacterial activity of the synthesized compounds

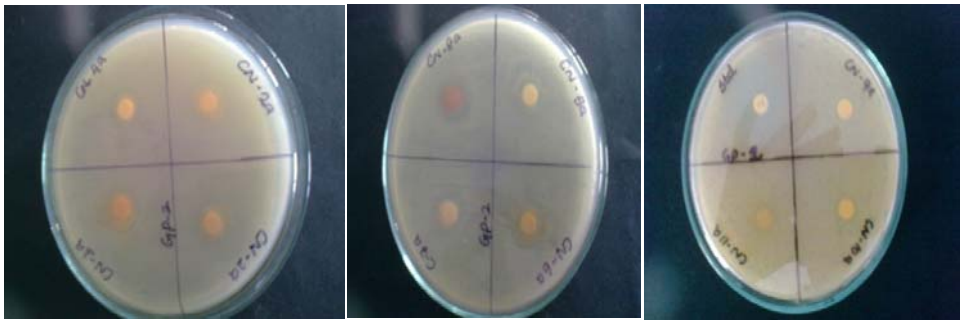
S. N	Micro organisms	Zone of Inhibition (in mm) Compounds (100µg/disc)											
		CN 1a	**CN 2a**	**CN 3a**	**CN 4a**	**CN 5a**	**CN 6a**	**CN 7a**	**CN 8a**	**CN 9a**	**CN 10a**	**CN 11a**	**Std ***
1	*Micrococcus Luteus*	10	8	9	9	10	9	9	10	11	11	10	18
2	*Staphylococcus Aureus*	11	10	12	8	11	12	12	11	10	11	11	17
3	*Bacillus Subtilis*	9	9	10	9	9	10	10	9	9	9	10	15
4	*Corynebacterim Diphtheria*	9	10	10	8	9	8	8	10	10	10	9	16
5	*Bacillus Linctus*	8	10	8	10	10	11	11	11	9	9	11	18
6	*Escherichia Coli*	11	10	12	11	10	11	11	11	13	15	12	16
7	*Pseudomonas aureginosa*	9	10	10	8	11	10	9	11	10	8	11	15
8	*Rhodosporum Rubrum*	11	12	12	11	11	12	12	14	13	14	12	15
9	*Vibrio cholera*	9	11	11	10	14	11	13	11	14	10	15	18
10	*Salmonella Paratyphi*	11	11	13	11	10	9	13	11	10	9	11	15

*- Ciprofloxacin

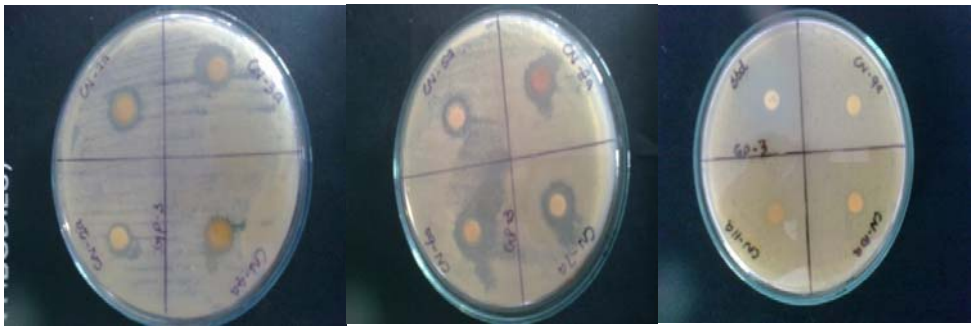
ANTI-BACTERIAL SCREENING



Micrococcus luteus

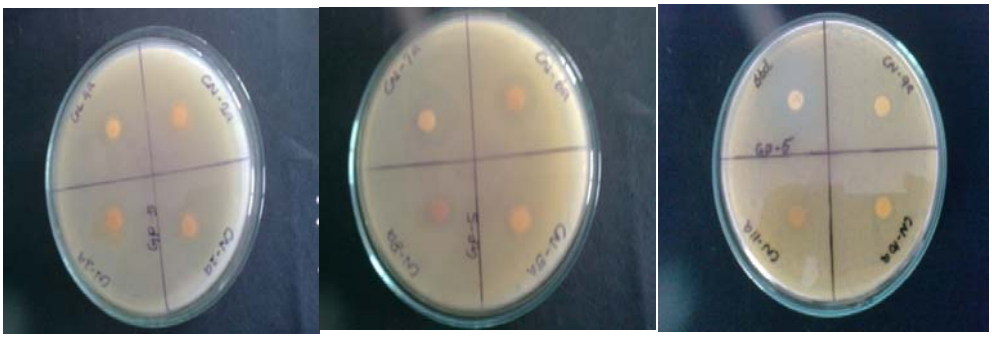


Staphylococcus aureus



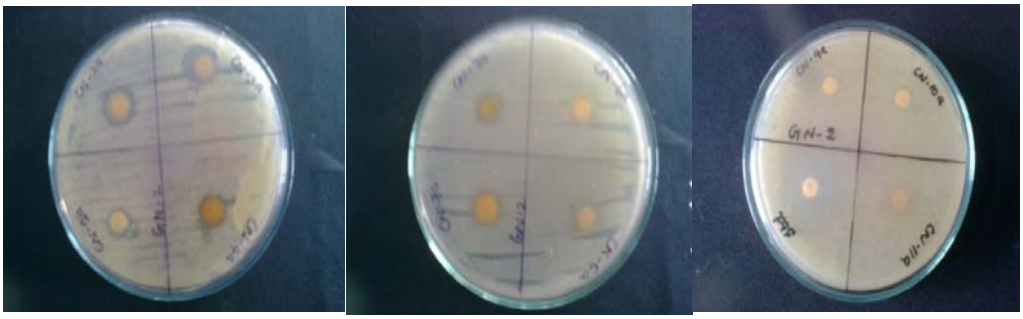
Bacillus subtilis

Corynebacterium diphtheria

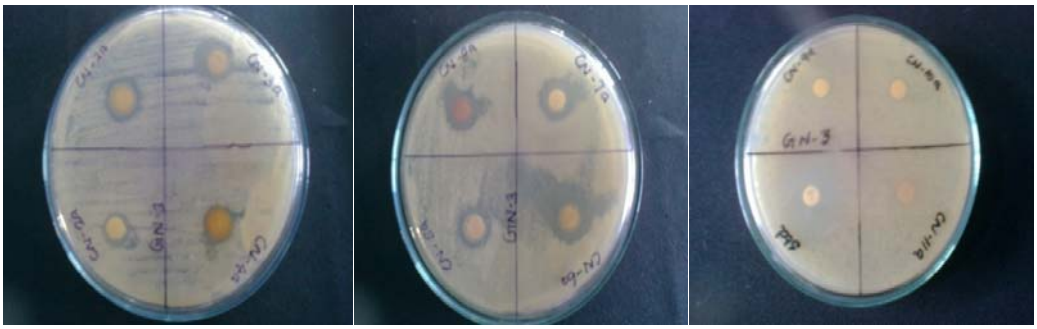


Bacillus linctus

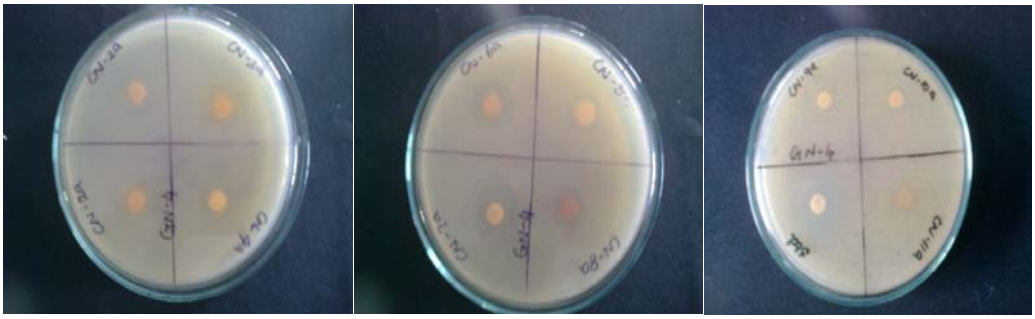
Escherichia coli



Pseudomonas aureginosa



Rhodosporum rubrum



Vibrio cholerae

Salmonella paratyphi

SERIAL DILUTION METHOD

Table 6: MIC values of the synthesized compounds.

SN	Micro organisms	MIC VALUES (µg/ml)											
		CN 1a	CN 2a	CN 3a	CN 4a	CN 5a	CN 6a	CN 7a	CN 8a	CN 9a	CN 10a	CN 11a	Std*
1	*M.luteus*	1.2	5	2.5	2.5	1.2	2.5	2.5	1.2	1.2	2.5	1.2	1.2
2	*S.aureus*	1.2	2.5	1.2	5	2.5	1.2	1.2	2.5	2.5	2.5	1.2	1.2
3	*B.subtilis*	5	5	2.5	2.5	2.5	2.5	1.2	1.2	2.5	2.5	1.2	1.2
4	*C.diphtheriae*	2.5	1.2	1.2	5	2.5	5	5	1.2	1.2	1.2	2.5	1.2
5	*B.linctus*	5	2.5	5	2.5	2.5	2.5	1.2	1.2	2.5	2.5	1.2	1.2
6	*E.coli*	1.2	2.5	1.2	2.5	1.2	5	1.2	1.2	1.2	2.5	2.5	1.2
7	*P.aureginosa*	2.5	2.5	1.2	2.5	1.2	2.5	2.5	2.5	1.2	1.2	2.5	1.2
8	*R.rubrum*	5	2.5	2.5	5	2.5	1.2	2.5	1.2	2.5	2.5	1.2	1.2
9	*V.cholerae*	5	2.5	2.5	5	1.2	2.5	1.2	2.5	1.2	2.5	1.2	1.2
10	*S.paratyphi*	2.5	2.5	1.2	2.5	2.5	5	1.2	2.5	2.5	2.5	2.5	1.2

*-Ciprofloxacin

ANTI-FUNGAL SCREENING

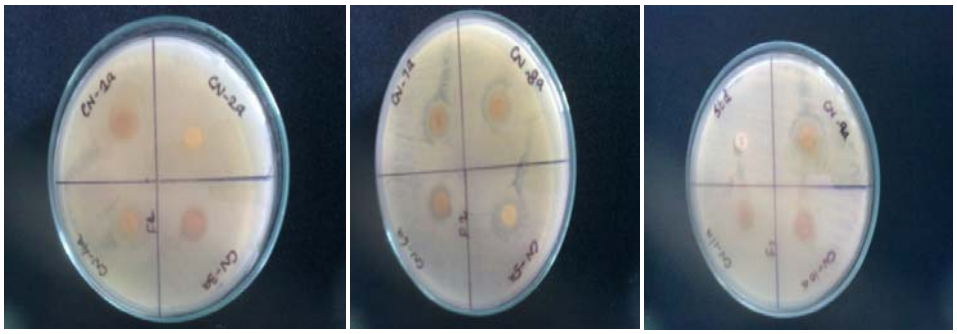
DISC DIFFUSION METHOD

Table 7: Anti-fungal activity of the synthesized compounds

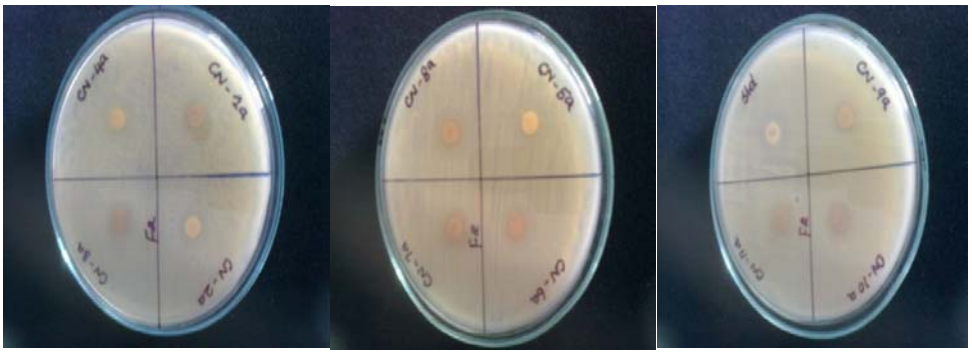
S. N	Micro organisms	Zone of inhibition (in mm) Compound (100 µg/disc)											
		CN 1a	CN 2a	CN 3a	CN 4a	CN 5a	CN 6a	CN 7a	CN 8a	CN 9a	CN 10a	CN 11a	Std*
1	*Candida albicans*	10	9	9	9	11	8	8	10	10	11	9	11
2	*Streptomyces griseus*	10	7	8	8	9	6	6	7	8	8	9	10
3	*Aspergillus niger*	9	9	9	8	10	9	11	10	9	9	8	11
4	*Aspergillus fumigalis*	8	8	7	7	9	7	7	8	8	7	6	9
5	*Monascus ruber*	8	7	7	6	8	8	6	7	7	8	8	9

*- Clotrimazole

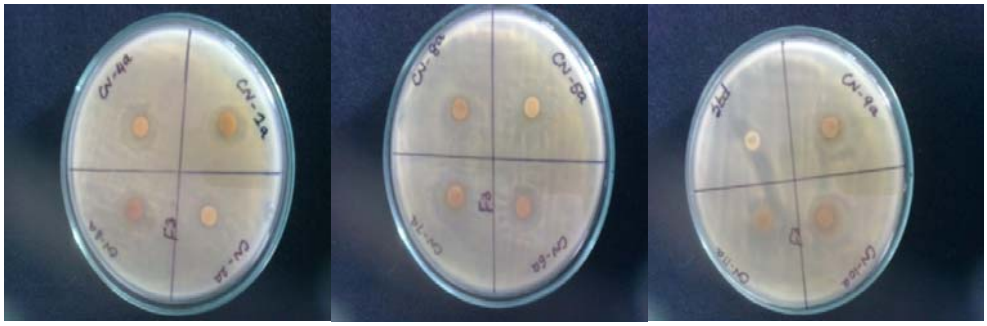
ANTI-FUNGAL SCREENING



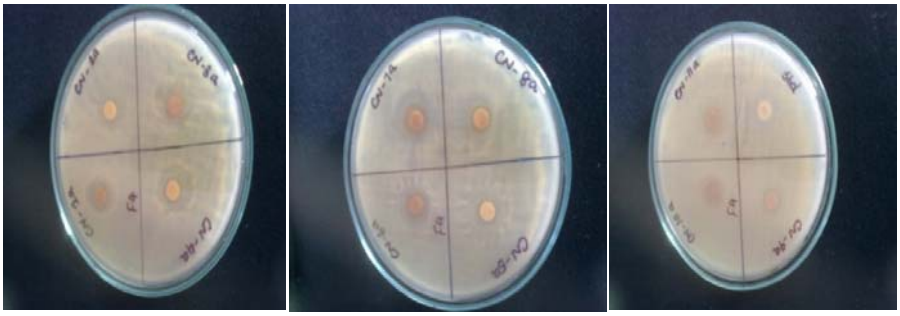
Candida albicans



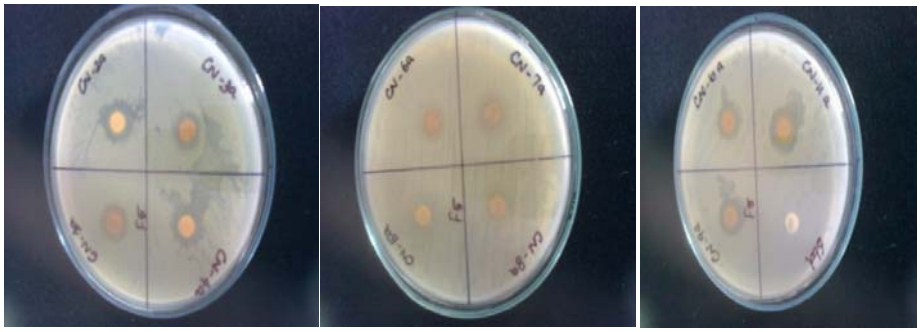
Streptomyces griseus



Aspergillus niger



Aspergillus fumigatus



Monascus ruber

SERIAL DILUTION METHOD

Table 8: MIC Values of the synthesized compounds

S.N	Micro organisms	MIC VALUES (µg/ml)										
		CN 1a	CN 2a	CN 3a	CN 4a	CN 5a	CN 6a	CN 7a	CN 8a	CN 9a	CN 10a	CN 11a
1	*Candida albicans*	2.5	1.2	2.5	2.5	2.5	5	5	2.5	2.5	2.5	1.2
2	*Streptomyces griseus*	1.2	2.5	2.5	2.5	2.5	5	2.5	2.5	5	2.5	1.2
3	*Aspergillus niger*	2.5	2.5	2.5	5	2.5	1.2	1.2	2.5	2.5	2.5	2.5
4	*Aspergillus fumigatus*	5	5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	5	1.2
5	*Monascus ruber*	2.5	2.5	2.5	2.5	2.5	1.2	1.2	2.5	5.0	2.5	2.5

RESULT AND DISCUSSION

Provoked by the biological activity of the Cinnoline and in view of ongoing search for the most potent anti-malarial, anti-tubercular and anti-microbial agent, some novel 3, 7 Di substituted derivatives of Cinnoline have been synthesized and their anti-malarial, anti-tubercular and anti-microbial activity studied**.**

The synthesis of designed Cinnoline analogues involves a five step procedure from a commercially available starting material

Step 1: This step involves diazotisation of Sulphanilamide using Sodium nitrite in Hcl at a temp at 0-5^{o}C to form diazonium chloride salt of Sulphanilamide (primary aromatic amine) in 95% yield.

Step 2: This step involves Griess diazo reaction as the formed diazonium chloride salt of Sulphanilamide reacts with acetyl acetone and ethanol to form a keto group of Phenyl hydrazano acetyl acetone-4-sulfonamide further it tautomerised to give the enol form, in 92% yield.

Step 3: This step involves intramolecular cyclization of enol form of Phenyl hydrazano acetyl acetone-4-Sulfonamide by using Polyphosphoric acid as a cyclising agent at a temp of 60-65^{o}C, in 76% yield.

Step 4: This step involves the attack of methyl proton in the acetyl group by a base (40% NaOH) which results in the formation of carbanion. It reacts with substituted aromatic aldehydes which undergoes protonation by using ethanol to form Cinnoline based chalcones (CN-1-CN-11), in 82% yield.

Step 5: This step involves intermolecular cyclization of Cinnoline based chalcones by reaction with hydrazine hydrate and acetic acid to form 4-methyl-3-[5-(substituted phenyl)-4,5-dihydro-1H-Pyrazol-3-yl]cinnoline-6-Sulfonamide (CN-1a – CN-11a) by losing a water molecule, the yield was found to be 86%.

All the derivatives of Cinnolines were obtained with good yield. The percentage yield was found to be in the range of 74% - 92%.

CHARACTERIZATION OF SYNTHEZISED COMPOUNDS

The physical parameters like, molecular weight, solubility, melting point, and R_f of the synthesized compounds were determined.

The melting points of the synthesized compounds determined and were uncorrected, and melting range was found to be between, 80-205^{0}C

The purity of the synthesized compounds was established by single spot on the TLC Plate and all the compounds found to be pure.

The structures of the synthesized compounds were confirmed by IR, NMR, and mass spectral analysis.

IR Spectrum of the synthesized compounds showed the characteristic absorption band at 1503.24 cm^{-1}, 681.713cm^{-1}, 1582.31cm^{-1} arising from stretching vibration of bands (C=N,C-S,C=C respectively), 1530.24cm^{-1}, 1401.03cm^{-1} due to NO_2 stretching, C-Cl stretching, 680.749cm^{-1}, 810.17 cm^{-1}, 750cm^{-1} due to the substitution of benzene ring (O,P,M-substituted benzene), due to 3200cm^{-1} of R_2NH confirms the chemical structure of the compounds synthesized.

PMR spectra of the synthesized Cinnoline derivatives shows 4 aromatic protons as a multiplet in 8.21-8.63 ppm, 2 Protons of amino group appeared at 2.0 ppm as a single, and 3 Protons of methyl group appeared as a singlet in 2.35-2.55 ppm, 4 protons of aromatic group appeared as a multiplet in 6.68-6.95 ppm. Thus the proton magnetic spectrum of the compound was in full agreement with its molecular formula, with regard to proton count and the chemical shift also.

The Mass Spectral analysis of the synthesized compounds CN-I and CN-1a was performed, and the mass spectrum of the compound was in agreement with its molecular weight.

BIOLOGICAL SCREENING

ANTI-MALARIAL ACTIVITY:

The anti-malarial studies was carried out with all synthesized Cinnoline derivatives in the concentration of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml in DMSO against *Plasmodium falciparum* by using Candle jar method. Pyremethamine of same concentrations were used as a standard. The anti-malarial activities of the compounds were evaluated by estimation of percentage of inhibition of parasitemia at different concentration. It could be seen that these newly synthesized derivatives of Cinnoline exhibit moderate to good anti-malarial activity. Out of the compounds synthesized, CN-5a was most potent which exhibit 87% inhibition at 80µg/ml concentration. Other derivatives,which also showed inhibition more than 50% respectively.

ANTI -MYCOBACTERIAL ACTIVITY

The synthesized compounds CN-2a and CN-5a were tested for activity against *Mycobacterium tuberculosis* H_{37}Rv using Resazurin assay method at the concentration of 1µg/ml, 10µg/ml and 100µg/ml in DMSO. Rifampicin 1µg/ml was used as a standard. The anti-mycobacterial activity of the synthesized compounds was evaluated by the change in colour of resazurin from Pink to blue by Oxido reductase with in viable cell. Among these two compounds, compound CN-5a inhibited the growth of *Mycobacterium* at 100µg/ml.

ANTI-BACTERIAL ACTIVITY

The antibacterial studies were carried out with all the synthesized Cinnoline derivatives against gram positive and gram negative bacteria. It could be seen that these newly synthesized derivatives of Cinnoline exhibit moderate to good anti-bacterial activity. Out of the compounds synthesized, CN-11a, CN-9a, CN-5a was most potent with zone of inhibition against *Rhodosporum rubrum, Vibrio cholera* and *Escherichia coli*.

The MIC of the synthesized compounds against *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Corynebacterium diphtheria, Bacillus linctus, Escherchia coli, Pseudomonas aureginosa, Rhodosporum rubrum, Vibrio cholera* and *Salmonella paratyphi* was determined by serial dilution method, was found to be in the range of 1.2-2.5µg/ml.

ANTI- FUNGAL ACTIVITY

Evaluation of the results from anti-fungal studies showed that synthesised Cinnoline derivatives exhibits moderate to good anti-fungal activity against *Candida albicans Aspergillus fumigatus, Streptomyces griseus, Aspergillus niger*, *Aspergillus fumigalis*, *Monascus ruber* with zone of inhibition was found to be in the range of (6-11mm). Out of the compounds, CN-5a, CN-7a, CN-11a were showed good activity against *Candida albicans, Streptomyces griseus*, with zone of inhibition was found to be in the range of 11mm.

The MIC of the synthesized compounds against *Candida albicans Aspergillus fumigalis, Streptomyces griseus, Aspergillus niger*, *Aspergillus fumigalis*, *Monascus ruber* was determined by serial diluton method, was found to be in the range of 1.2-2.5µg/ml.

All the synthesized compounds have showed good Anti-malarial, Anti-microbial activity and compound CN-5a shown Anti-tubercular activity, due to the incorporation of Sulfanamido and Pyrazole groups into Cinnoline nucleus. Out of these synthesized compounds CN-5a was found to be a promising candidate which exhibits Anti-malarial, Anti-tubercular and Anti-microbial activities due to the synergistic action of the substituents such as methoxy (OCH_3) and hydroxyl (OH) group in CN-5a along with incorporation of Sulfanamido and Pyrazole in Cinnoline nucleus.

Conclusion

CONCLUSION

In Summary, some novel substituted Cinnoline derivatives have been synthesized and evaluated for its anti-malarial, anti-tubercular and anti-microbial activity. All derivatives demonstrated significant anti-malarial, anti-tubercular and anti-microbial activity amongst, compound CN-5a was found to be most potent compound with promising activity against resistant strains of *Plasmodium falciparum*, *Mycobacterium tuberculosis* H_{37}Rv, bacteria and fungus.

Taking into account the significant activities of the examined compounds, it is believed that further optimization of these identified chemical leads can probably lead to the development of more active molecules. Further studies on its possible mechanism and in vivo trials in experimental animals to broaden their pharmacological assessment, may provide a new analogue that can overcome drug resistance, prolonged treatment, complex drug regimen and side effects involved in the treatment of infectious diseases.

Bibliography

REFERENCES

1. Sir Deker Barton, *Comprehensive organic Chemistry*, 1993, Vol 2, 724-726.
2. Wakein, L.P.G Waring , *Comprehensive organic Chemistry*, 1990, Vol 2, 702-703.
3. Gareth Thomas. *Medicinal Chemistry*, 5th edition. John Willey & Sons Ltd; 2003.
4. Tisler, M and Stanovic, *Advance in Heterocyclic Chemistry*, 1970, 408.
5. Matyus. P, Czako K. *Trend in heterocyclic Chemistry*, 1993, Vol 2, 3, 249.
6. Katrizky, Alan R, Charles W. Rees, *Comprehensive heterocyclic Chemistry*, 1990, Vol 3, 22.
7. Stanczak A. & W. Pakulska, *Pharmazi*e 2001, 56(6), 501.
8. Alexander L. Ruche man, Sudhir K. Singh, Abhijit Ray, Xiaohua Wu,Jin-Ming, *Bioorg .Med. Chem*. .2004, 12, 795–806.
9. Ken ford, J.R.; Simpson, J.C.E, *J. Chem. Soc.* 1967, 20, 917–920.
10. Edwards JW, *Survey of Ant malarial Drugs* 1976, ***11,*** 946.
11. James D. Chesney, *Bulletin of the world health organisation*, 1981, 59(5), 495-462.
12. Cigarillo, G., Grella, G., Loriga, M., Curzu, M. M., Schiatti, *G. Farmaco [Sci],* 1978, 33(11), 866-74.
13. Sayed, G. H, Sayed, M. R, Shaaban, *Egypt. J. Chem*. 2002,45, 767- 776.
14. Barraja, P, Diana, P, Lauria, A., Passannanti, A, Almerico, A.M.; Minnei, *Bioorg. Med. Chem.*1999, **7,** 1591–1596.
15. Vingkar, S.L.; Bobade, A.S.; Khadse, B.G. *Indian J. Chem.* 1993, *32B,* 1281– 1285. Anu Agarwal, Kumkum Srivastava, S. K. Puri, S. Sinhac and Prem M. S. Chauhana, *Bioorg .Med. Chem. Lett* ,2005, 15 , 5218–5221
16. Mavurapu Satyanarayana , Wei Feng , Liang Cheng , Angela A. Liu , Yuan-Chin Tsai , Leroy F. Liu , *Bioorg .Med. Chem. Lett*.,2008, 16, 7824–7831.
17. Coudert, P. Duroux, E. Bastide, *J. Pharm Belg*.1991, 46 (6), 375-80.
18. Kassab, R. Myers, *Egypt. J. Chem*. 2002, 45, 1055-1073.
19. Mojahidul, I., Anees, *Acta Poloniae Pharmaceutica,* 2008,65 (3), 353-362.
20. Strappaghetti, G., Corsano, *J. Med. Chem*. 2001, 44, 2118.
21. Pham, H. C., Lasserre, B., Pham, H. C. A., Palhares de, M. A. L., Tronche, P., Couquelet, C. *Prostaglandins Leukot Essent Fatty Acids*.,1988:33(2) 143-147.
22. Cao, S., Qian, X., Song, G., Chai, B., Jiang, *J. Agric. Food Chem.* 2003, 51,152-155.
23. Willemot, *Plant Physiol*, 1997, 60(1), 1-4.
24. Mohammad Asif1, Anita Singh2, International *Journal of ChemTech Research* 2010, Vol.2, 1112-1128.
25. Youssef, A. S., Marzouk, M. I., Madkour, H. M. F., El-Soll, A. M. A., El-Hashash, *Can. J. Chem*. 2005, 83, 251-259.

26. Sato, Y.Zhao, *J.Synt. Org. Chem*, 2007, 21, 695-706.
27. Laura, K., Wing, H. A., Behanna, L. J., Van, E. D., Martin, W., and Hantamalala, *Current Alzheimer Research* ,2006, 3, 205
28. Alvarado, M.; Barcelo, M.; Carro, *Chem. Biodivers,* 2006, *3*, 106–117.
29. Cherng, S. C., Huang, W. H., Shiau, C. Y., Lee, A. R., Chou, T. C.,*Eur. J .Pharmacol*. 2006, 532, 32-37.
30. Dogruer, Sahin, M. F., Kupeli, E., Yesilada, E. *Turk. J. Chem.* 2003, 27,727-738.
31. Ma*i, IlFarmaco,* 1995, 40, 921-929.
32. Schatz, F.; Wagner-Jauregg, *Helv. Chim. Acta,* 1968, *51*, 1919–1931.
33. Deniz, S. D. M. and Fethi, S. *Turk J Chem*, 2007, 27,727–738.
34. Frolov, E. B., Lakner, F. J., Khvat, *Tetrahedron Lett*. 2004, 45, 4693-4696.
35. Shephered G. and J.L. Fedrick., *Advances in Heterocyclic Chemistry*, Vol 4, 1965, 353.
36. Robert E. Lyle and Paul. Anderson *Advances in Heterocyclic Chemistry,* Vol.9, 1991 PP. 84-85.
37. Mitsumori T., Sedo J., Werdi F.,*Chem mater*, 2003, 15,3579.
38. Jan K.G., Danieru A., Piitaa F.H..*J.Am.Chem.Soci,* 2002, 124, 13463.
39. Nunn A.J., Schofield K., *J.chem.Soc,*1953,373, 3700-3706.
40. O. V. Vinogradova , Balova, *Chemistry of Heterocyclic Compounds,* Vol. 44, 2008, 5.
41. Kumar, S.; Bawa, S.; Drabu, *Molbank, 2009*, 12, M 618.
42. Neber P. W., Knoller G., Herrst K., Trissler, *J.Am.Chem.Soci,* 2002, 1241, 435-438.
43. Alford E. J. and Schofield K., *J. Chem. Soc.*, 1952,323, 2102-2107.
44. Gomaa M. A.M., *Tetrahedron Lett.* 2003, 44, 3493.
45. Barber H. J., Lunt E., *Tetrahedron Lett*. 2003, 56, 8933-8937.
46. Turck A., Guoquiner S.G., *Tetrahedron*, 1995, 51, 13045.
47. Menon R.G, Purushothaman E., *J. Ind. Chem. Soci,* 1993, 70, 553.
48. Menon R.G, Purushothaman E., *J. Ind. Chem. Soci,* 1995, 72,731.
49. Menon R.G, Purushothaman E., *Ind. J. Chem.*, 1996, 35B, 1185.
50. Vuye A., Arzeim-Forsch, 1983, 33(12), 1623.
51. Abbady, MS, Radwan Sh Mr. Bakhite EA, *Ind. J. Chem,* 1993, *32B, 1281*.
52. Purushothaman.E and Vijayaraj KK, *Ind. J. Heterocycl. Chem.*, 1999, 14B, 43.
53. Abdel.W.Lewgoud M., *Arch.pharm*, 2007, 33, 340-345.
54. Pilugin J.F., *Tetrahedron Lett,* 1999, 40,677.
55. Narqud L.V.G, Badiger V.V., Yarnal S.M., *J.Pharm. Sci.* 1992, 81,365-366.
56. Padhmanand Sudhakar and Prasanth Subramani, Vol 16, Issue 01 Jan 2007.
57. Endis K, Sina B, Marchesini , *Am J Trop Med Hyg* 64 (1-2 Suppl): 2001, 97–106.
58. Escalante A, Ayala F. *Proc Natl Acad Sci USA* 1994, 91 (24), 11373–7.

59. *WebMD*, Malaria symptoms Last Updated: May 2007,16-20
60. Wells TN et al. New Medicines to improve control & contribute to the eradication of malaria. Net Rev Drug Discovery 8(11); 879-91(2009).
61. Arhurst DC, Williams JE , *J Clin Pathol* 49 (7) 1996, 533–38.
62. Minjian Chen, Huixian Gan & Heinz G. Remold. The Journal of Immunology, 2006, 176; 3707-3716.
63. Alaa. A. El- Tombary,Khadiga A. Ismail, Mervatz El. Azzouni, Synthesis & antitoxoplasmosis effect 2nd Farmaco 54(1999) 486-495.
64. Porth CM. *Alterations in respiratory function: respiratory tract infections, neoplasms, and childhood disorder*s. In Porth CM, Kunert MP. *Pathophysiology: Concepts of Altered Health States. Philadelphia,* PA: Lippincott Williams & Wilkins; 2002:615-619.
65. Marjorie P, Golden,M.D.Holenarasipur, Mayo Clinic, Scottsdale, Arizona. *Extrapulmonary Tuberculosis: An Overview*
66. Nahid P, Pai M, Hopewell P. *Proc. Am. Thorac .Soc.* 2006, 3 (1), 103–10.
67. *American Thoracic Society and Centres for Disease Control and Prevention. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med.* 2000; 161(4 pt 1): 1376-1395.
68. Korf JE, Pynaert G, Tournoy K, et al. *Macrophage reprogramming by mycolic acid promotes a tolerogenic response in experimental asthma. Am J Respir Crit Care Med.* 2006; 174(2): 152-160.
69. Nicod LP. *Immunology of tuberculosis*. Swiss Med Wkly. 2007; 137(25-26): 357-362.
70. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. *Tuberculosis. Lancet*. 2003;362: 887-899.
71. Van Crevel R, Ottenhoff THM, Van der Meer JWM. *Innate Immunity to Mycobacterium tuberculosis. Clin Microbial Rev*. 2002; 15: 294-309.
72. Jensen PA, Lambert LA, Iademarco MF, Rizdon R; *Centres for Disease Control and Prevention. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings*, 2005. MMWR Recomm Rep. 2005; 54(RR-17): 1-141.
73. Ferguson JS, Wies JJ, Martin JL, Schlesinger LS. *Complement protein C_3 binding to Mycobacterium tuberculosis is initiated by the classical pathway in human bronchoalveolar lavage fluid. Infect Immun*. 2004; 72: 2564-2573.
74. Lee RB, Li W, Chatterjee D, Lee RE. Rapid structural characterization of the arabinogalactan and lipoarabinomannan in live mycobacterial cells using 2D and 3D HR-MAS NMR: Structural changes in the arabinan due to ethambutol treatment and gene mutation are observed. Glycobiology. 2005; 15(2): 139-151.
75. Satoskar, R.S.Bhandarkar & Anaipure S.P, *Pharmacology & Pharmacotherapeutics*, 16th edition, *Popular Prakashan,* Mumbai, 611-683.

76. Mutlu G, Mutlu E, Bellmeyer A, Rubinstein I. *Am. J. Med* .2006, 119 (8) 639–46.
77. Bacteria (eubacteria)-Taxanomy Browser, Ret; 2008-09-16.
78. Orter JR, *Bacteriological Reviews* 1976, 40 (2), 260–9.
79. Yonath A, Bashan A. "Ribosomal Crystallography: initiation, peptide bond formation, and amino acid polymerization are hampered by antibiotics". Annu Rev Microbiol 58: 2004; 233-51.
80. Khachatourians GG. "Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria". CMAJ 159(9): 1998; 1129-36.
81. Woods GL, Walker D,*Clinical Microbiology Reviews* 1996, 9 (3) ,382–404.
82. A fungus Oxford Dictionaries Ret; 2011-02-26.
83. Richer V..*chem.Ber* 1983, 16, 677-683.
84. Wieslawa Lawgowd, *Bioorg .Med. Chem Lett,* 18, 2007, 3570-3572.
85. Lourie E.M., Morley J.S., Simpson J.C.E.,. *Bit. J. Pharmacol*. 1951, 6, 643
86. Kenfroad J.R, Simpson J.C,E, *J.Chem.soc*,1947,917-920.
87. Nidhi Gautham, chourasia V, *Ind.J. Chem.* June 2010, 49B. 830-835.
88. Vladimir T. Abaev, Alexander V. Butinb, *Tetrahedron* 2000,56, 8933-8937
89. Jacobs T.L., *Heterocyclic compounds Wiley, New York*, 1957, chapter 6, pp 43-42
90. Hipparagi S.M, Nargund L.V.G., *Ind. J. Heterocycl. Chem,* 2003, 13,123-126.
91. Saravanan G., Abbady M.S, *Ind.J. Chem, 2006*, *42B, 1281-1283.*
92. Conrad R.A., White W.A., US4379929. 1983 [*Chem. Abstr.* 1983, 99, P22481].
93. Lowrie H.S., US 3272818. 1966 [*Chem.Abstr.* 65, 1966, P18599].
94. Chung-Kyu Ryu, Jung yoon lee, *Bioorganic & Medicinal Chemistry Lett*, Vol 16; Issue 7, April 2006, pp 1850-1853.
95. Anshu J., Akhar, Indian *J. Chem.* 2006, 45B, 1704.
96. Vikas, Darbahamullae, Molbank, 2009, m-656.
97. Sandhya Bawa, Rajiv Kumar, Gita Chawla, Suresh Kumar, *Molbank ,* 2010, m-688.
98. Miyamoto K., Matsumoto J., Nakamura S., JP 62228067. 1987 *[Chem. Abstr.* 1988, 108, P221710].
99. Gasc J.C., Humbert D., Hunt P. F., FR 2549833. 1985 [*Chem.Abstr.* 1985, 103, P123498].
100. Ravindra Reddy, Praden D.J, *Ind Chem Soci*; 70: 1993; 553.
101. Ramalingam P, Ganapaty S, Ch.Babu Rao, Ravi TK. *Ind J Hetero Chem* 2006; 15; 359-62.
102. Preethi, Kagthara, Tejas upadhyay, Rajeev Doshi & Prakash, 2000; 10, 09-12.
103. Yamazaki T., Draper R.E., Castle R.N., *J.Hetero Chem*. 1978., 15, 1039-1040.
104. Barbara Stefanska, Edward Borowski, Maria M. Bioorganic & Medicinal Chemistry, Vol 13, Issue 6, 15 March 2005, PP 1969-1975.

105. Alexander L. Ruchelman, Sudhir K. Singh, Abhijit Ray, Nai Angela Liu, Leroy F. Liu and Edmond J. Lavoiea. *Bioorganic Med Chem*. 2004, 12, 795-806.
106. Wei Feng, Mavuurapu Satyanarayana, Yuan-chin Tsai, Angela A. Liu, *Bioorganic Med Chem*, 2008, 18, 3570-3572.
107. Girolomo cirrinicione, Anna Maria Almerio, Paola, *J.Med Chem*, 1933, 140-145.
108. Olga. Vinogra,. Viktor Sorokoumov, *Bioorg Med Chem*; 2009, 18, 2564-2572.
109. Schenker E., US 4478837. 1984 [*Chem. Abstr*. 1985, 103, P37488].
110. Lowrie H.S., US 3239526. 1966 [*Chem. Abstr*. 1966, 64, P17616].
111. Jpn. Kokai Tokkyo, JP 61280483. 1986 [*Chem. Abstr*. 1988, 108, P131839].
112. Boyle J., Terence A., GB 2160201. 1986 [*Chem. Abstr*. 1986, 105, P208903].
113. Allais A., Rousseau G., Meier J., Nomine G., *Chim. Ther*. 1973, 8, 154-168.
114. Lowrie H.S., *J. Med. Chem*. 1966, 9, 664-669.
115. Kikazawa K., Hiiragi M., Ishimaru H., [*Chem. Abstr*. 1988, 109, P110445].
116. Gilman D.J., Ger 2151487. 1972 [*Chem. Abstr*. 1972, 77, P34545].
117. Nunn A.J et al *J.Chem.Soc*. 1965, 1936-1942.
118. Dorn H., DD 247219. 1988 [*Chem. Abstr*. 1988, 108, P131841].
119. Furniss B.S., Hannaford A.J., *Vogels text book of practical organic chemistry*, fifth edition. 2009.
120. Robert M. Silverstein, *Spectrometric identification of organic compounds*, Sixth edition, 2007.
121. Culturing Plasmodium falciparum. Standard Operating Procedure University of Edinburgh 2007.
122. James Wiloms, *Experimental parasitology* 1972, 12, 274-322.
123. Kirstenmoll, IngerLjungstorm, ArturScherf, Mats Wahlgren, *Malaria research and reference reagent resource centre Manasas*, USA. 2008, 1-321.
124. James B.Jensen, William Trager. *The Journal of parasitology*. 1987, 63, 883-886.
125. Sanjib Bhattacharya et al, *Int. J. Chem. Tech* Res. 2009, 1(2). 67-70.
126. Manju Bala, Krishna Ray & S.M. Gupta. *Indian J Med* Res. 2005, 122, 48-51.
127. *IP* 1996- Appendix 9, *Microbiological assays and tests.*
128. Vingkar, S.L., Bobade, A.S., Khadse., *Indian. J. Chem*. 1993, 32B, 1281-1284.
129. Kumar A., Sinha, S. Chauhan, S. *Bioorg. Med. Chem. Lett.* 2002, 12, 667.
130. Collins, L.A., Franzblan, *Antimicrob. Agents. Chemother*. 1997, 41, 1004.
131. www.Wikepedia.com